FIGURE 1A

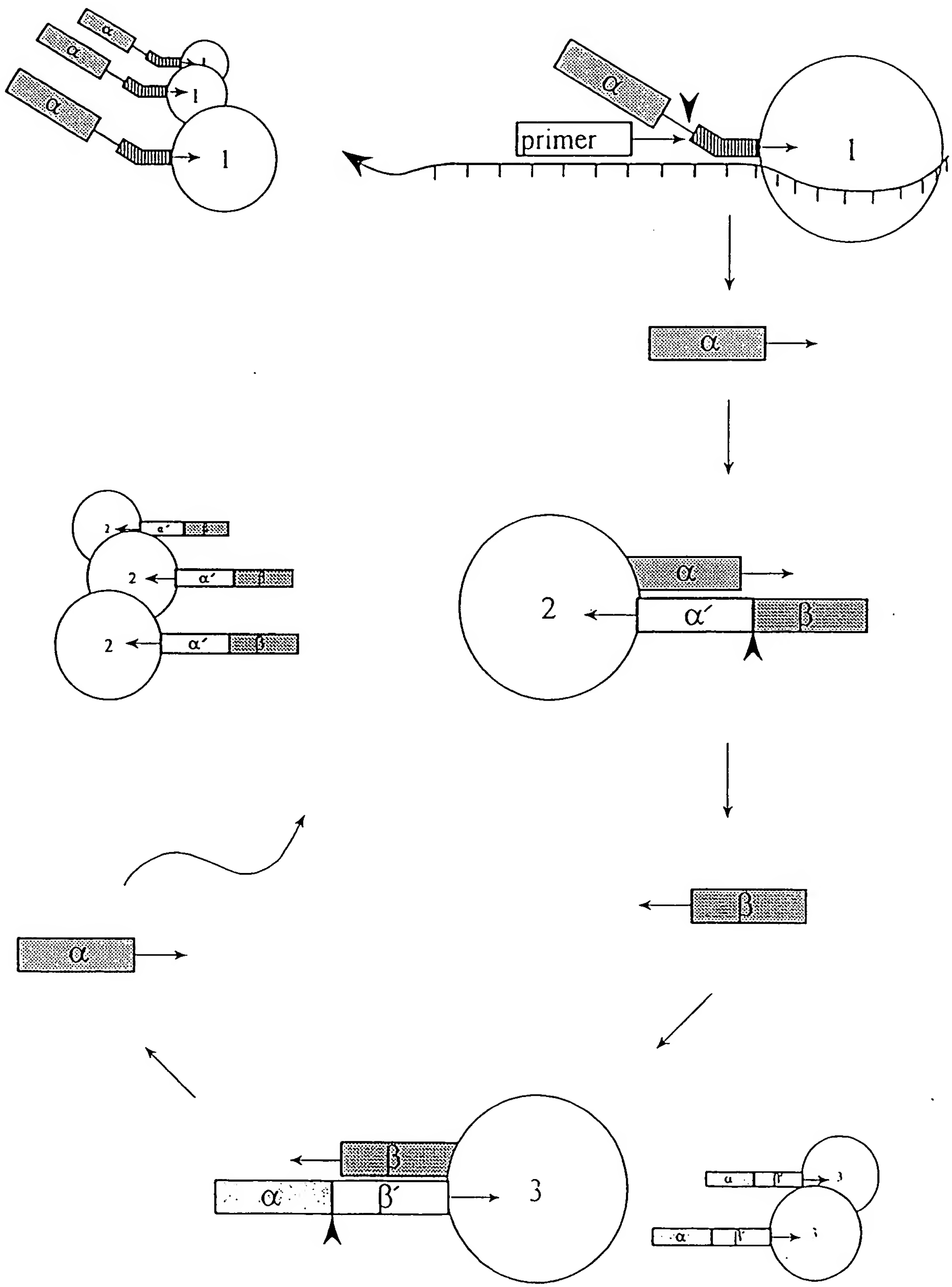
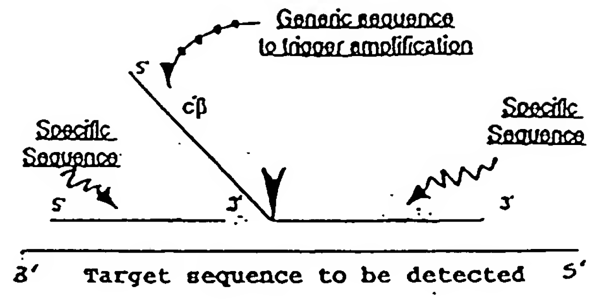


FIGURE 1B

PART ONE: TRIGGER REACTION



PART TWO: DETECTION REACTION

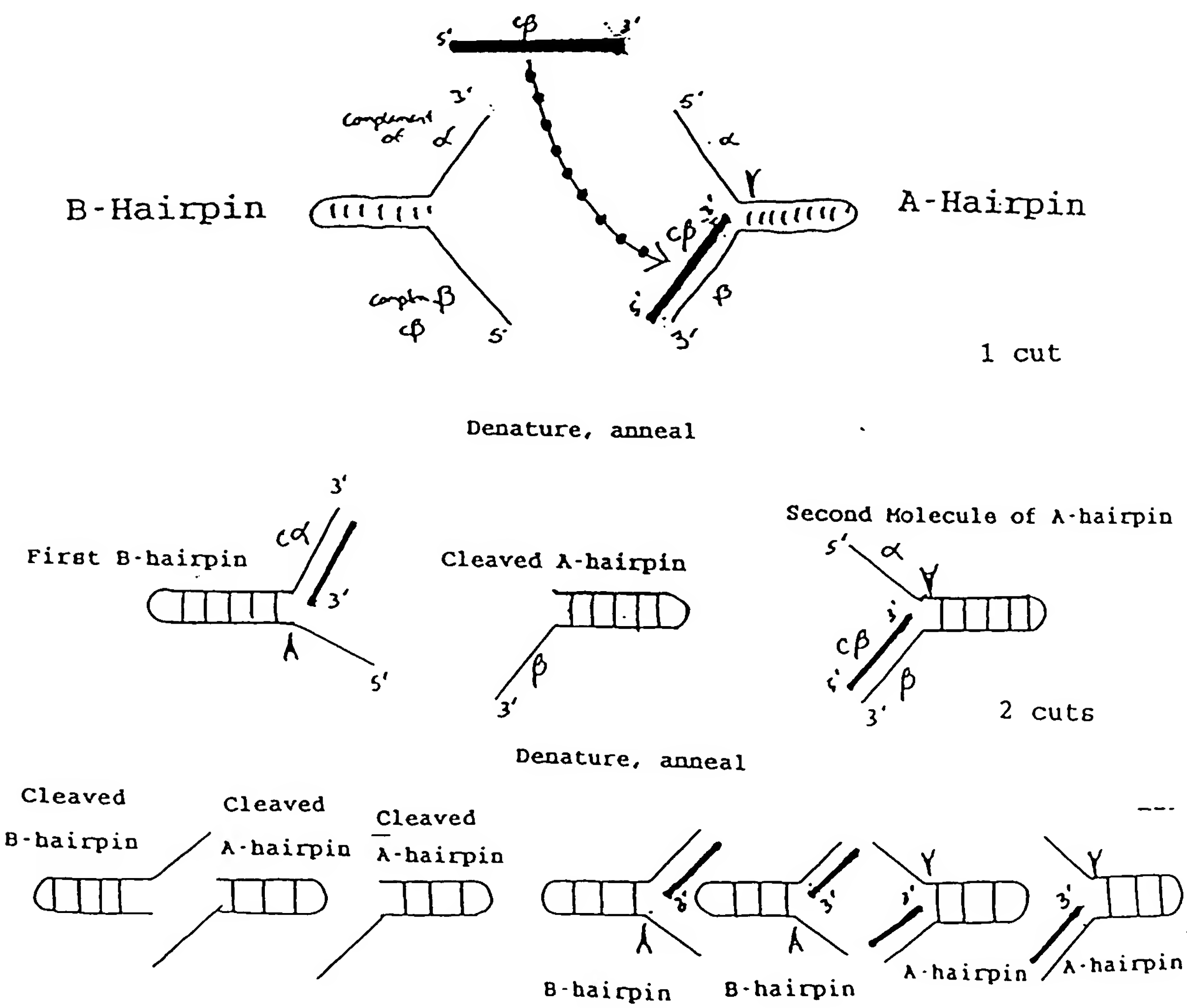


FIGURE 2

```
MAJORITY (SEQ ID NO:7)  ATCXXGGCCATGCTTCCCCTCTTTGAGCCCAAAGGCCGGGTCCTCCTGGTGGACGGCCACCACCTGGCCT

ONAPTAO  (SEQ ID NO:1)  ...AG..G......G.................G.....................................  70
DNAPTFL  (SEQ ID NO:2)  ......................................C..G............................  67
DNAPTTH  (SEQ ID NO:3)  ...GA............G........A...........................................  70

MAJORITY                ACCGCACCTTCTTCGCCCTGAAGGGCCTCACCACCAGCCGGGCGAACCGGTGCAGGCGGTCTACGGCTT

ONAPTAO                 ...........CA..............................G..G.......................  140
DNAPTFL                 .............T.....C....................C........C..T.................  137
DNAPTTH                 ..................................G...................................  140

MAJORITY                CGCCAAGAGCCTCCTCAAGGCCCTGAAGGAGGACGGGGACXXGGCGGTGXTCGTGGTCTTTGACGCCAAG

ONAPTAO                 ........................C........................A....................  207
DNAPTFL                 ......A.................................GT..T.........................  204
DNAPTTH                 .....................................T..AA...C..CT....................  210

MAJORITY                GCCCCCTCCTTCCGCCACGAGGCCTACGAGGCCTACAAGGCGGGCCGGGCCCCCACCCCGGAGGACTTTC

ONAPTAO                 ............................G..GG.......................G.............  277
DNAPTFL                 ......................................................................  274
DNAPTTH                 ............................................GA.......G.....C........C.  280

MAJORITY                CCCGGCAGCTCGCCCTCATCAAGGAGCTGGTGGACCTCCTGGGGCTTGCGCGCCTCGAGGTCCCCGGCTA

ONAPTAO                 .......A......................................G.................G.....  347
DNAPTFL                 ..........G...............T.............A..C...T...G..G.....T.......T  344
DNAPTTH                 ............................................T..A.C....................  350
```

3

FIGURE 2 (cont'd)

```
MAJORITY  (SEQ ID NO:7)  CGAGCCGGACGACGTXCTGGCCACCCTGGCCAAGAAGGCGGAAAAGGAGGGGTACGAGGTGCGCATCCTC

DNAPTAQ   (SEQ ID NO:1)  ...............C.......G...........................C........C.........  417
DNAPTFL   (SEQ ID NO:2)  T..............G..................CG..................................  414
DNAPTTH   (SEQ ID NO:3)  ...............T..C...................................................  420

MAJORITY                 ACCGCCGACCGCGACCTCTACCAGCTCCTTTCCGACCGCATCGCCGTCCTCCACCCCGAGGGGTACCTCA

DNAPTAQ                  .........AAA.....T........................CA..........................  487
DNAPTFL                  ..T.............................G..G.........A..........T...........G.  484
DNAPTTH                  .......................A...G.C.........G......................CC......  490

MAJORITY                 TCACCCCGGCGTGGCTTTGGGAGAAGTACGGCCTGAGGCCGGAGCAGTGGGTGGACTACCGGGCCCTGGC

DNAPTAQ                  ..........C...........A.................C..C.......CC...............A.  557
DNAPTFL                  ..................AC...............C.C................................  554
DNAPTTH                  .........A........................C......................T...C.....C.T  560

MAJORITY                 GCGGGACCCCTCCGACAACCTCCCCGGGGTCAAGGGCATCGGGGAGAAGACCGCCCXGAAGCTCCTCXAG

DNAPTAQ                  C......GAG...........T.............................G..GAG......T..GG..  627
DNAPTFL                  ............G..T...A..........G.........................A..G....A..CGC  624
DNAPTTH                  ........................................................TC.........A..  630

MAJORITY                 GAGTGCGGGAGCCTGGAAAACCTCCTCAAGAACCTGGACCGGGTGAAGCCCGC···CXTCCGGGAGAAGA

DNAPTAQ                  ..................GC......................C..........···.A............  694
DNAPTFL                  ........................T..C..C.........A..........T.···.T.G.........C  691
DNAPTTH                  ........A...................................A.....A.AAAA.G............  700
```

FIGURE 2 (cont'd)

```
MAJORITY  (SEQ ID NO:7)  TCCAGCCCCACATGGAXGACCTGAXGCTCTCCTGGGAGCTXTCCCAGGTGCCCACCGACCTGCCCCTGGA

DNAPTAQ   (SEQ ID NO:1)  ...T............C..T....A............C..GG..A.........................  764
DNAPTFL   (SEQ ID NO:2)  .......GGG......G.C....GCC..T...C..A....T..........A...T..............  761
DNAPTTH   (SEQ ID NO:3)  ..A........C....A.....C.G........T......C....G...............C........  770

MAJORITY                 GGTGGACTTCGCCAAGXGGCGGGAGCCCGACCGGGAGGGGCTTAGGCCTTTCTGGAGAGGCTGGAGTTT

DNAPTAQ                  ...............AA....................A.........................T......  834
DNAPTFL                  ...........GG.G.C.C..CACA...A...T......T..GC....T...T.....C..T........  831
DNAPTTH                  .......C.....C..G..................................C.................C  840

MAJORITY                 GGCAGCCTCCTCCACGAGTTCGGCCTCCTGGAGGGCCCCAAGGCCCTGGAGGAGGCCCCCTGGCCCCCCC

DNAPTAQ                  ..........................T.....AA....................................  904
DNAPTFL                  ..A................................G..G.....GGCA....................T.  901
DNAPTTH                  ..................................C....GCCC...........................  910

MAJORITY                 CGGAAGGGGCCTTCGTGGGCTTTGTCCTTTCCCGCCCCGAGCCCATGTGGGCCGAGCTTCTGGCCCTGGC

DNAPTAQ                  .........................G.........AAG.................T..............  974
DNAPTFL                  ..........T..TT........TC.T.......T...................................  971
DNAPTTH                  ......................C.....C.......................G......AAA........  980

MAJORITY                 CGCCGCCAGGGAGGGCCGGGTCCACCGGGCACCAGACCCCTTTAXGGGCCTXAGGGACCTXAAGGAGGTG

DNAPTAQ                  ...........G..................C..C..G..T.A..AA.C...C........G.......C.  1044
DNAPTFL                  T.GG..GT.......G..CC....T.......A.......C...G......G........T....G....  1041
DNAPTTH                  ....TG......C........G.........G..........GGC...G..A.A......C........C  1050
```

5

202T20" 82E4Z00T

FIGURE 2 (cont'd)

```
MAJORITY  (SEQ ID NO:7)  CGGGGXCTCCTCGCCCAAGGACCTGGCCGTTTTGGCCCTGAGGGAGGGCCTXGACCTCXTGCCCGGGGACG

DNAPTAQ   (SEQ ID NO:1)  .....G..T........A......AG....C.............A.....T.G....CC......C....
DNAPTFL   (SEQ ID NO:2)  .....AA....G..............G............C..........G......T.C..A.A.....  1111
DNAPTTH   (SEQ ID NO:3)  .....C.................C.....C......TC.........G..A......G............  1120

MAJORITY                 ACCCCATGCTCCTCGCCTACCTCCTGGACCCCTCCAACACCACCCCCGAGGGGTGGCCCGGCGCTACGG

DNAPTAQ                  ...............................T......................................  1184
DNAPTFL                  .............G........T.......................T.................T.....  1181
DNAPTTH                  ..........................................................G...........  1190

MAJORITY                 GGGGAGTGGACGGAGGAXGCGGGGGAGCGGGCCCTCCTXTCCGAGAGGCTCTTCCXGAACCTXXXGGAG

DNAPTAQ                  C.................G................GC..T...............GCC.....GTG..G.  1254
DNAPTFL                  ..................T.........A..........GG.....C.C.......A..C...AAA....  1251
DNAPTTH                  ..................C..C.CCC.C...........C..G.........CAT.G......CCTTA..  1260

MAJORITY                 CGCCTTGAGGGGGAGGAGAGGCTCCTTTGGCTTTACCAGGAGGTGGAGAAGCCCCTTTCCCGGGTCCTGG

DNAPTAQ                  A.G..................................G...........G..........GCT.......  1324
DNAPTFL                  ......A....A..A..AC.C..G............G................G...........GT...  1321
DNAPTTH                  .....C.............A............C.....C........A........C.............  1330

MAJORITY                 CCCACATGGAGGCCACGGGGGTXCGGCTGGACGTGGCCTACCTCCAGGCCCTXTCCCTGGAGGTGGCGGA

DNAPTAQ                  ......................G..C..............T...AG....T.G..............C..  1394
DNAPTFL                  ...GG.................C.............................C.............A..C  1391
DNAPTTH                  ................C.....A....................T........T.........C.T.....  1400
```

6

FIGURE 2 (cont'd)

```
MAJORITY (SEQ ID NO:7)  GGAGATCCGCCGCCTCGAGGAGGAGGTCTTCCGCCTGGCCGGCCACCCCTTCAACCTCAACTCCCCGGAC
ONAPTAQ  (SEQ ID NO:1)  .......GC...........CC............................................C...  1464
DNAPTFL  (SEQ ID NO:2)  ....G.G....AG..G......................................................  1461
DNAPTTH  (SEQ ID NO:3)  ..................................T....G..............................  14

MAJORITY                CAGCTGGAAAGGGTGCTCTTTGACGAGCTXGGGCTTCCCGCCATCGGCAAGACGGAGAAGACXGGCAAGC
ONAPTAQ                 ..............C..............A................................C.......  1534
DNAPTFL                 ........GC...................G..C..G..T.......................G..G..A.  1531
DNAPTTH                 .............................TA...........T.G..G......C.A.....A.......  1540

MAJORITY                GCTCCACCAGCGCCGCCGTGCTGGAGGCCCTXCGXGAGGCCCACCCCATCGTGGAGAAGATCCTGCAGTA
ONAPTAQ                 ...................C...........C..C...................................  1604
DNAPTFL                 .............T.................G..A....................CCGC...........  1601
DNAPTTH                 ................G..............A..G.............................C...C.  1610

MAJORITY                CCGGGAGCTCACCAAGCTCAAGAACACCTACATXGACCCCCTGCCXGXCCTCGTCCACCCCAGGACGGGC
ONAPTAQ                 ..................G....G.........T......T....G.A....A.................  1
DNAPTFL                 .................................A...........C.C...G..........A...C...  16
DNAPTTH                 ...............................C.G........C..AAG............C.........  16

MAJORITY                CGCCTCCACACCCGCTTCAACCAGACGGCCACGGCCACGGCAGGCTTAGTAGCTCCGACCCCAACCTGC
ONAPTAQ                 ...............................................A...........T........C.  1744
DNAPTFL                 ..G.............................C...............TCC...................  1741
DNAPTTH                 .........................................G............................  1750
```

7

FIGURE 2 (cont'd)

```
MAJORITY  (SEQ ID NO:7)  AGAACATCCCCGTCCGCACCCCXCTGGGCCAGAGGATCCCCCGGCCCTTCGTGCCCGAGCAGCCXTGCGT

DNAPTAQ   (SEQ ID NO:1)  ......................G..T..G......................A.C...........G...C  1814
DNAPTFL   (SEQ ID NO:2)  .............G........T..........C.C........A....................C....  1811
DNAPTTH   (SEQ ID NO:3)  ......................CT....................................C...T....C  182

MAJORITY                 GTTGGTGGCCCTGGACTATAGCCAGATAGAGCTCCGGGTCCTGGCCCACCTCTCCGGGGACGAGAACCTG

DNAPTAQ                  A.................................A....G..................C...........  1884
DNAPTFL                  .C......T.T.......C........T.....T....................................  1881
DNAPTTH                  ....................................C......C....................A.....  1890

MAJORITY                 ATCCGGGTCTTCCAGGAGGGGAGGGACATCCACACCCAGACCGCCAGCTGGATGTTCGGCGTCCCCCCGG

DNAPTAQ                  .....................C.............GG...............................G.  1954
DNAPTFL                  ...........T...................................................TT....C  1951
DNAPTTH                  ...A..................A......................A........................  1960

MAJORITY                 AGGCCGTGGACCCCCTGATGCGCCGGGCGGCCAAGACCATCAACTTCGGGGTCCTCTACGGCATGTCCGC

DNAPTAQ                  ....................................................................G.  20
DNAPTFL                  .A.GG..A.....T.......................................G................  202
DNAPTTH                  .....................................GG.G.........C...................  2030

MAJORITY                 CCACCGCCTCTCCCAGGAGCTTGCCATCCCCTACGAGGAGGCGGTGGCCTTCATTGAGCGCTACTTCCAG

DNAPTAQ                  .....................A........T............CCA.....................T..  2094
DNAPTFL                  .............GG.......T...............................................  2091
DNAPTTH                  ...TA.G.............................................T..A.............A  2100
```

8

FIGURE 2 (cont'd)

```
MAJORITY (SEQ ID NO:7)  AGCTTCCCCAAGGTGCGGGGCCTGGATTGAGAAGACCCTGGAGGAGGGCAGGAGCCGGGGTACGTGGAGA

DNAPTAQ  (SEQ ID NO:1)  ......................................................................  2164
DNAPTFL  (SEQ ID NO:2)  ....A.........................GG......C.........C.CC..........T.......  2161
DNAPTTH  (SEQ ID NO:3)  ..........................A..A.................G....A......C........A.  217?

MAJORITY                CCCTCTTCGGCCGCCGGCGCTACGTGCCCGACCTCAACGCCCGGGTGAAGAGCGTGCGGGAGGCGGCGGA

DNAPTAQ                 ................C...........A.....AG.G.............................C..  2234
DNAPTFL                 ......................T...................................C...........  2231
DNAPTTH                 ..........AA.AA........................................CA.......C.....  2240

MAJORITY                GCGCATGGCCTTCAACATGCCCGTCCAGGGCACCGCCGCCGACCTCATGAAGCTGGCCATGGTGAAGCTC

DNAPTAQ                 .........................................................T............  2304
DNAPTFL                 .....................G..........................................CG...T  2301
DNAPTTH                 ......................................................C...............  2310

MAJORITY                TTCCCCCGGCTXCAGGAAATGGGGGCCAGGATGCTCCTXCAGGTCCACGACGAGCTGGTCCTCGAGCCCC

DNAPTAQ                 ......A....GG.........................T...............................  23?
DNAPTFL                 ...........T......C.......G........TT.G.....G.........................  2371
DNAPTTH                 ........C..C.G...G.........C.C........C.................CC....C.......  2380

MAJORITY                CCAAAGAGCGGGCGGAGGXGGTGGCCGCTTTGGCCAAGGAGGTCATGGAGGGGGTCTATCCCCTGGCCGT

DNAPTAQ                 .A......A.........CC......CGGC.........................G..............  2444
DNAPTFL                 ....G..C.........AG...A..................................GG......CAG..  2441
DNAPTTH                 ..C...C......C....A......G................C.......AA..C.........C.....  2450
```

FIGURE 2 (cont'd)

```
MAJORITY (SEQ ID NO:7)  GCCCCTGGAGGTGGAGGTGGGGATGGGGGAGGACTGGCTCTCCGCCCAAGGAGTAG

DNAPTAQ  (SEQ ID NO:1)  ........................A.............................GA  2499
DNAPTFL  (SEQ ID NO:2)  .....................CC.................................  2496
DNAPTTH  (SEQ ID NO:3)  .......................................T..........GT....  2505
```

10

FIGURE 3

```
MAJORITY(SEQ ID NO:8)  MXAMLPLFEPKGRVLLVDGHHLAYRTFFALKGLTTSRGEPVQAVYGFAKSLLKALKEDG-DAVXVVFDAK

TAQ PRO (SEQ ID NO:4)  .RG........................H...................................I......  [illegible]
TFL PRO (SEQ ID NO:5)  .............................................................V.V......  [illegible]
TTH PRO (SEQ ID NO:6)  .E.........................................................YK..F......  70

MAJORITY               APSFRHEAYEAYKAGRAPTPEDFPRQLALIKELVDLLGLXRLEVPGYEADDVLATLAKKAEKEGYEVRIL

TAQ PRO                .........GG............................A..............S...............  139
TFL PRO                .......................................V......F...........R...........  138
TTH PRO                ......................................FT..............................  140

MAJORITY               TADRDLYQLLSDRIAVLHPEGYLITPAWLWEKYGLRPEQWVDYRALXGDPSDNLPGVKGIGEKTAXKLLX

TAQ PRO                ...K..........H......................D..A.....T..E...............R...E  209
TFL PRO                ...........E...I.............Y................A......I...........OR.IR  208
TTH PRO                .........V...V.......H....E...............F...V..................L...K  210

MAJORITY               EWGSLENLLKNLDRVKP-XXREKIXAHMEDLXLSXXLSXVRTDLPLEVDFAXRREPDREGLRAFLERLEF

TAQ PRO                ......A.......L..-AI....L...D..K..WD.AK.............K.......R.........  [illegible]
TFL PRO                ........FQH..Q...;SL...LQ.G..A.A..RK..Q.H..........GR..T.HL...........  277
TTH PRO                .................ENV....K..L...R..LE..R...........L.QG................  280

MAJORITY               GSLLHEFGLLEXPKALEEAPWPPPEGAFVGFVLSRPEPMWAELLALAAARXGRVHRAXDPLXGLRDLKEV

TAQ PRO                ...........S.......................K.....D........G......PE.YKA......A  348
TFL PRO                ...........G...A............L..SF..............G.WE..L...Q...R......G.  347
TTH PRO                ...........A.AP............................K....C.D......A...A..K.....  350
```

FIGURE 3 (cont'd)

```
MAJORITY (SEQ ID NO:8)  RGLLAKDLAVLALREGLDLXPGDDPMLLAYLLDPSNTTPEGVARRYGGEWTEDAGERALLSERLFXNLXX

TAQ PRO  (SEQ ID NO:4)  ........S........G.P................................E.....A......A..WG  [illegible]
TFL PRO  (SEQ ID NO:5)  ..I................F.E......................................A....QT.KE  [illegible]
TTH PRO  (SEQ ID NO:6)  ............S......V...................................AH........HR..LK  420

MAJORITY                RLEGEERLLWLYXEVEKPLSRVLAHMEATGVRLDVAYLQALSLEVAEEIRRLEEEVFRLAGHPFNLNSRD

TAQ PRO                 ............R...R...A.................R...........A...A...............  488
TFL PRO                 ..K.........E...........R....................EA.V.Q...................  487
TTH PRO                 ......K.....H...............................L.........................  490

MAJORITY                QLERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTKLKNTYIDPLPXLVHPRTG

TAQ PRO                 ......................................................S.......D.I.....  558
TFL PRO                 ..........................................DR..................A....K..  557
TTH PRO                 ..........R...L...Q...........................H..........V....S.......  560

MAJORITY                RLHTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFVAEEGWXLVALDYSQIELRVLAHLSGDENL

TAQ PRO                 ........................................I.....L.......................  6[illegible]
TFL PRO                 ..............................................V..V....................  627
TTH PRO                 ...........................................A..A.......................  630

MAJORITY                IRVFQEGRDIHTQTASWMFGVPPEAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIPYEEAVAFIERYFQ

TAQ PRO                 ............E.........R.......................................Q.......  698
TFL PRO                 .....................S..G...........................G..S..............  697
TTH PRO                 .......K............................V.................................  700
```

FIGURE 3 (cont'd)

```
MAJORITY (SEQ ID NO:8)  SFPKVRAWIEKTLEEGRRRGYVETLFGRRRYVPDLNARVKSVREAAERMAFNMPVQGTAADLMKLAMVKL

TAQ PRO  (SEQ ID NO:4)  ...........................................E.........................         76
TFL PRO  (SEQ ID NO:5)  .Y.......G............................................................R.      767
TTH PRO  (SEQ ID NO:6)  .................K.....................................................      770

MAJORITY                FPRLXEMGARMLLQVHDELVLEAPKXRAEXVAALAKEVMEGVYPLAVPLEVEVGXGEDWLSAKEX

TAQ PRO                 ....E....................E...A..R......................I.........         833
TFL PRO                 ....Q.L..................D...R............W..Q.........L.........         831
TTH PRO                 ....R..............L....QA...E........A..KA.............M........G       835
```

(3

FIGURE 4

Genes for Wild-Type and Pol(-) DNAPTaq

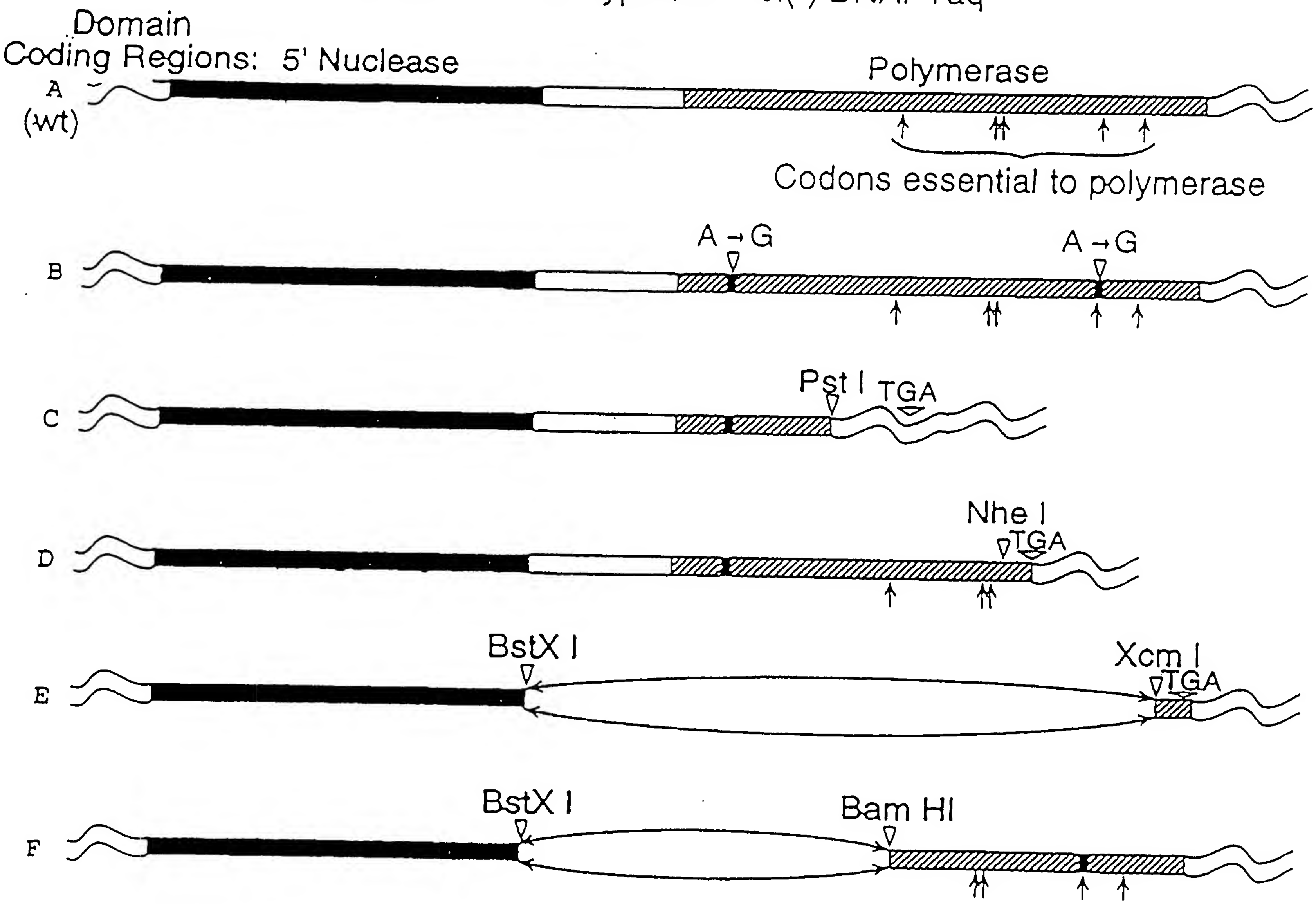


FIGURE 5

Genes for Wild-Type and Pol(-) DNAPTfl

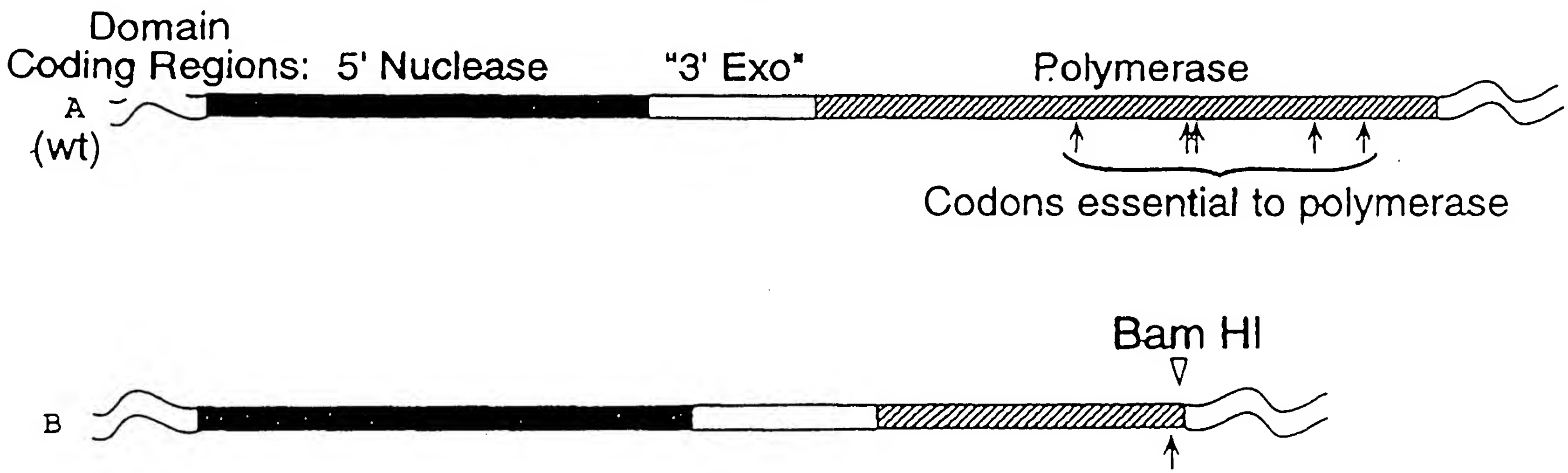


FIGURE 6

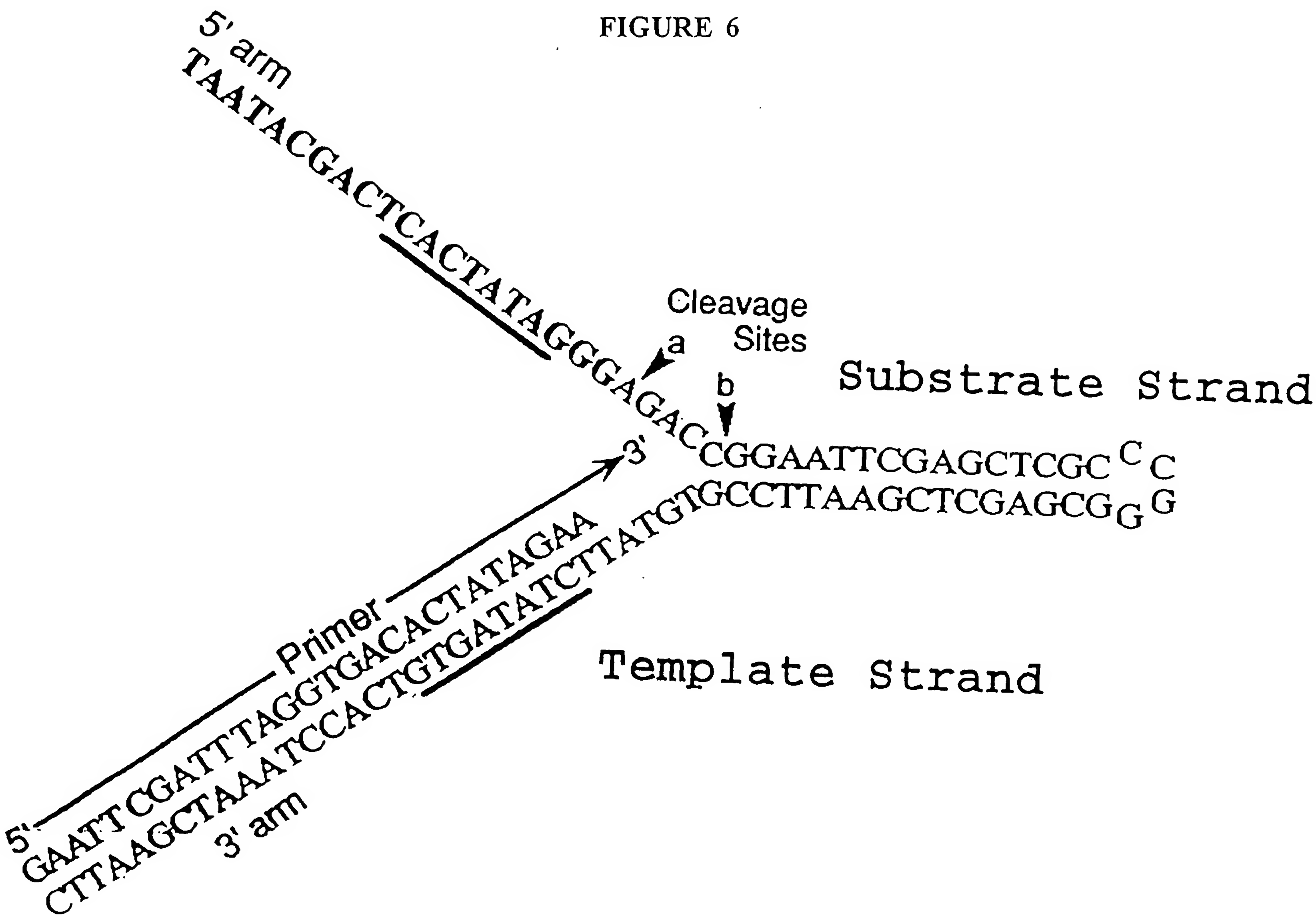
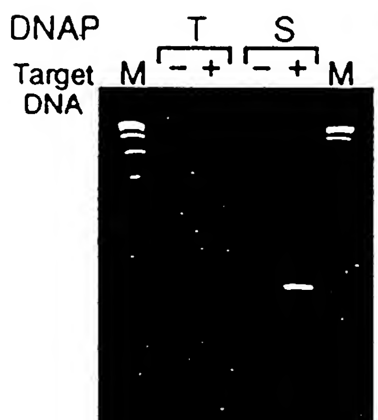

5' arm
TAATACGACTCACTATAGGGAGACC
Cleavage Sites
a
b
Substrate Strand
CGGAATTCGAGCTCGC C C
GCCTTAAGCTCGAGCG G G
3'
Primer
5' GAATTCGATTTAGGTGACACTATAGAA
CTTAAGCTAAATCCACTGTGATATCTTATGT
3' arm
Template Strand

FIGURE 7



17

FIGURE 8

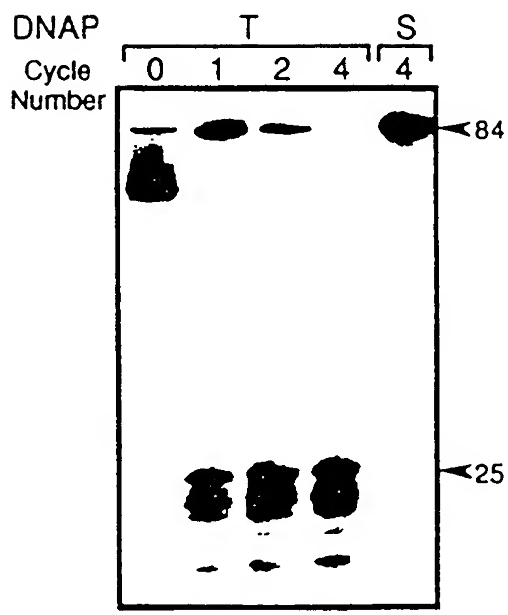
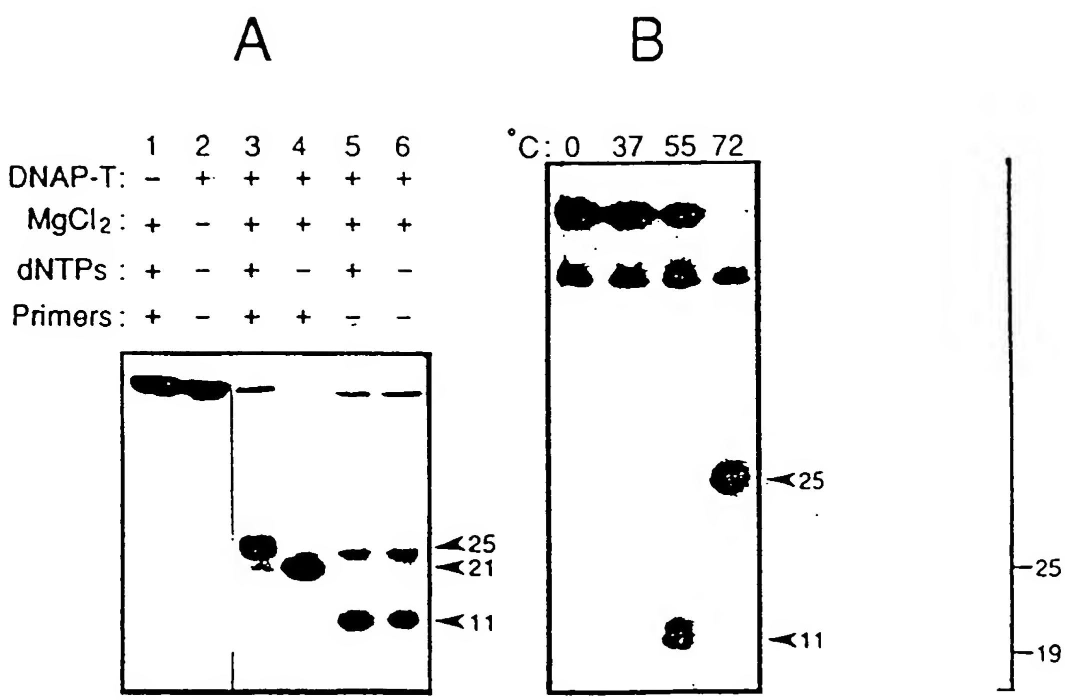


FIGURE 9



A
B
1 2 3 4 5 6
DNAP-T: – + + + + +
MgCl2 : + – + + + +
dNTPs : + – + – + –
Primers : + – + + – –
25
21
11
°C: 0 37 55 72
25
11
25
19

FIGURE 10

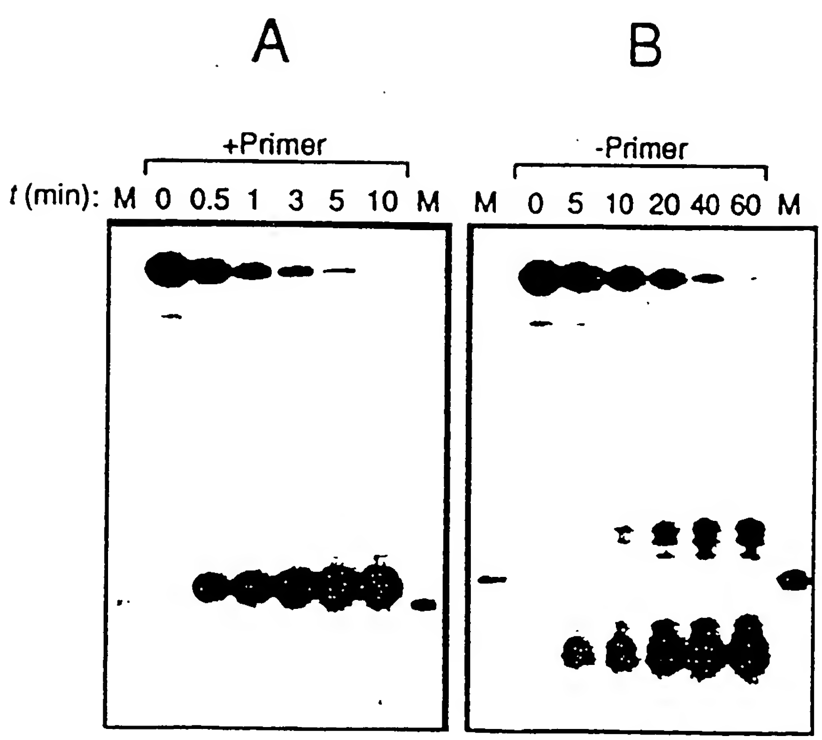


FIGURE 11

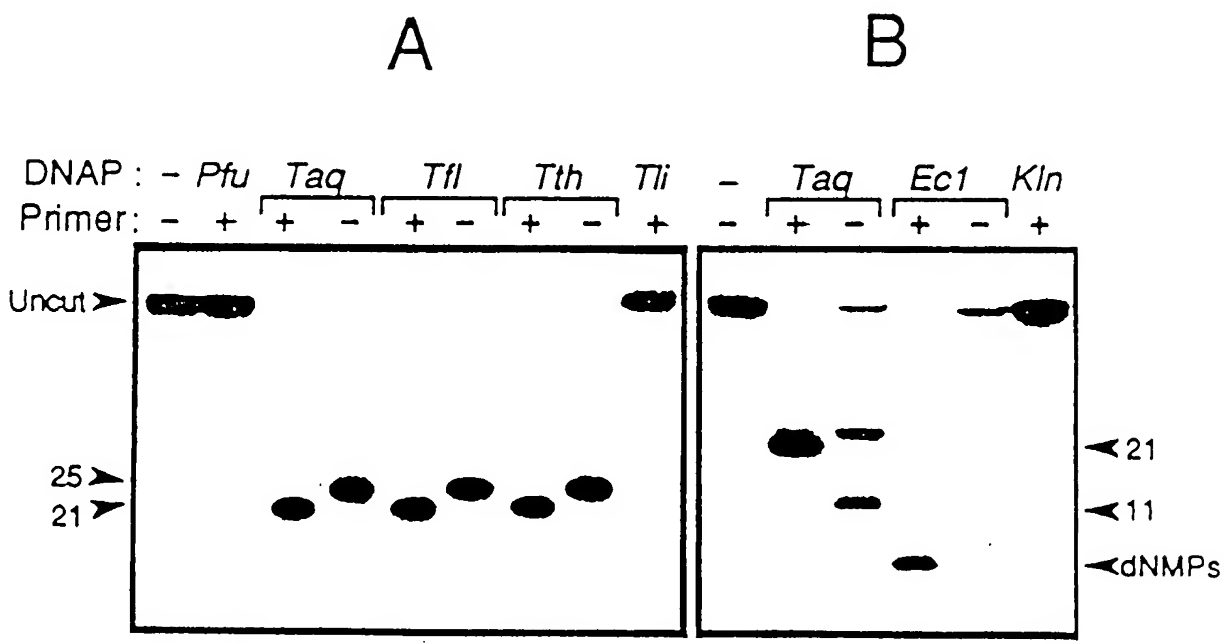

A
B
DNAP : – Pfu Taq Tfl Tth Tli
Primer : – + + – + – + – +
– Taq Ec1 Kln
– + – + – +
Uncut
25
21
21
11
dNMPs

FIGURE 12

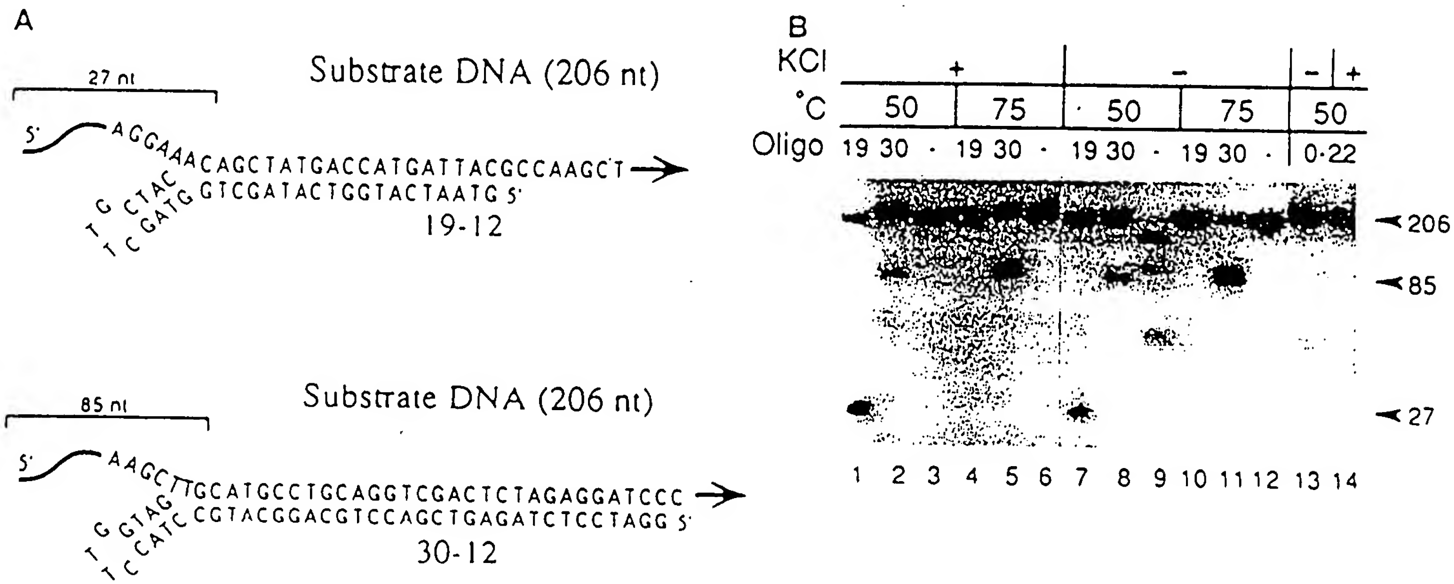
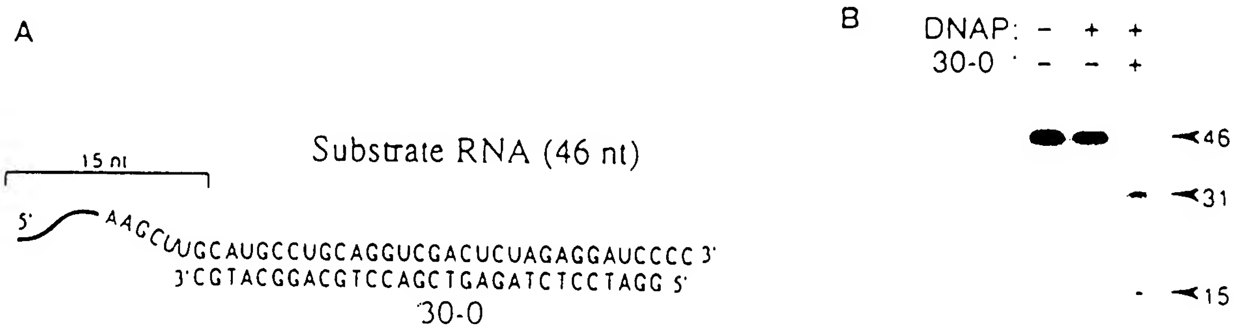
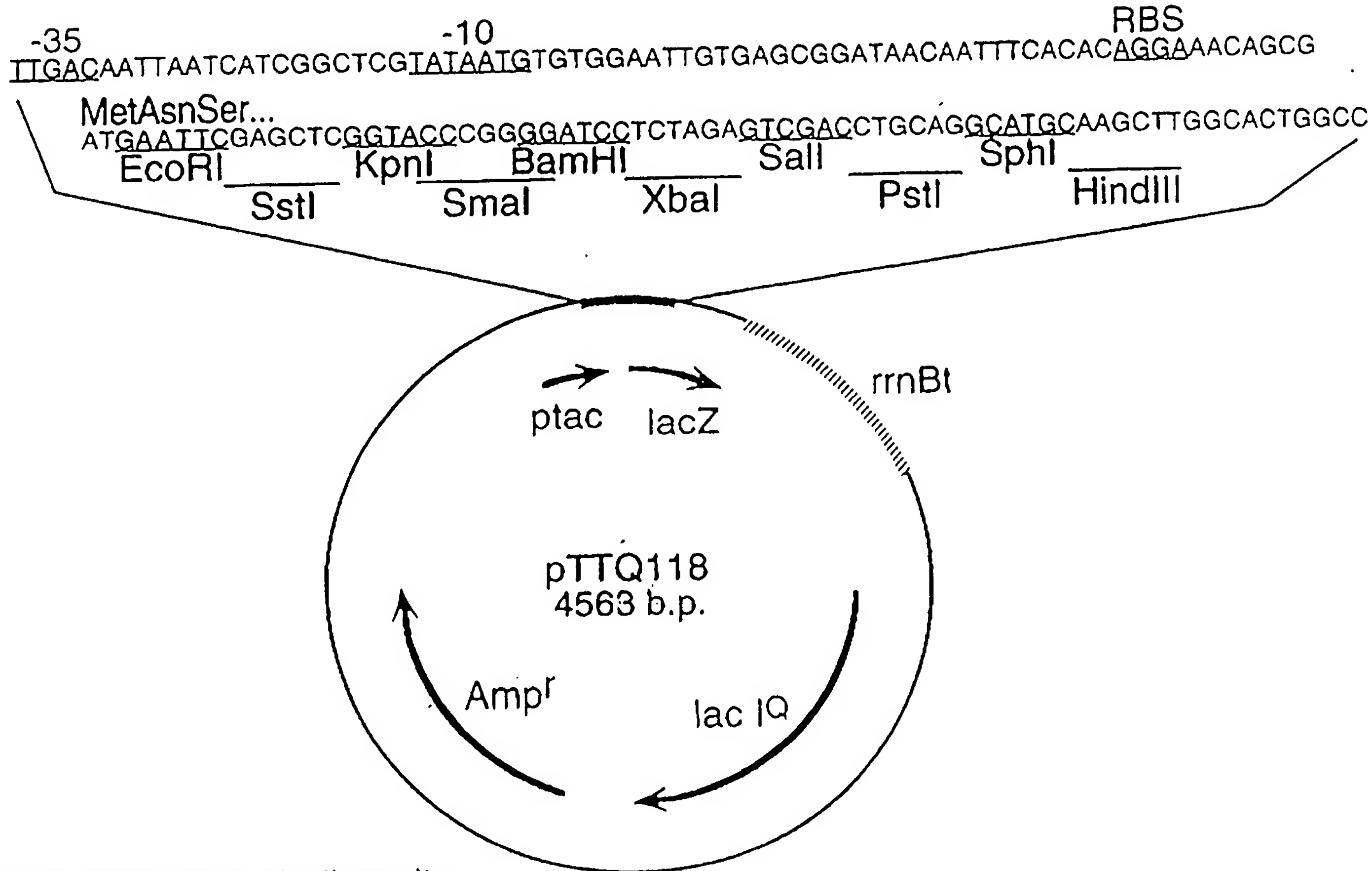

A
27 nt
Substrate DNA (206 nt)
5' AGGAAACAGCTATGACCATGATTACGCCAAGCT
TGCTACTCGATGGTCGATACTGGTACTAATG 5'
19-12
85 nt
Substrate DNA (206 nt)
5' AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCC
TGGTAGTCCATCCGTACGGACGTCCAGCTGAGATCTCCTAGG 5'
30-12
B
KCl + − − +
°C 50 75 50 75 50
Oligo 19 30 · 19 30 · 19 30 · 19 30 · 0·22
206
85
27
1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIGURE 13


A
15 nt
Substrate RNA (46 nt)
5' AAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCC 3'
3'CGTACGGACGTCCAGCTGAGATCTCCTAGG 5'
30-0
B
DNAP: - + +
30-0 - - +
46
31
15

23

FIGURE 14



RBS: Ribosome binding site

ptac: Synthetic tac promoter

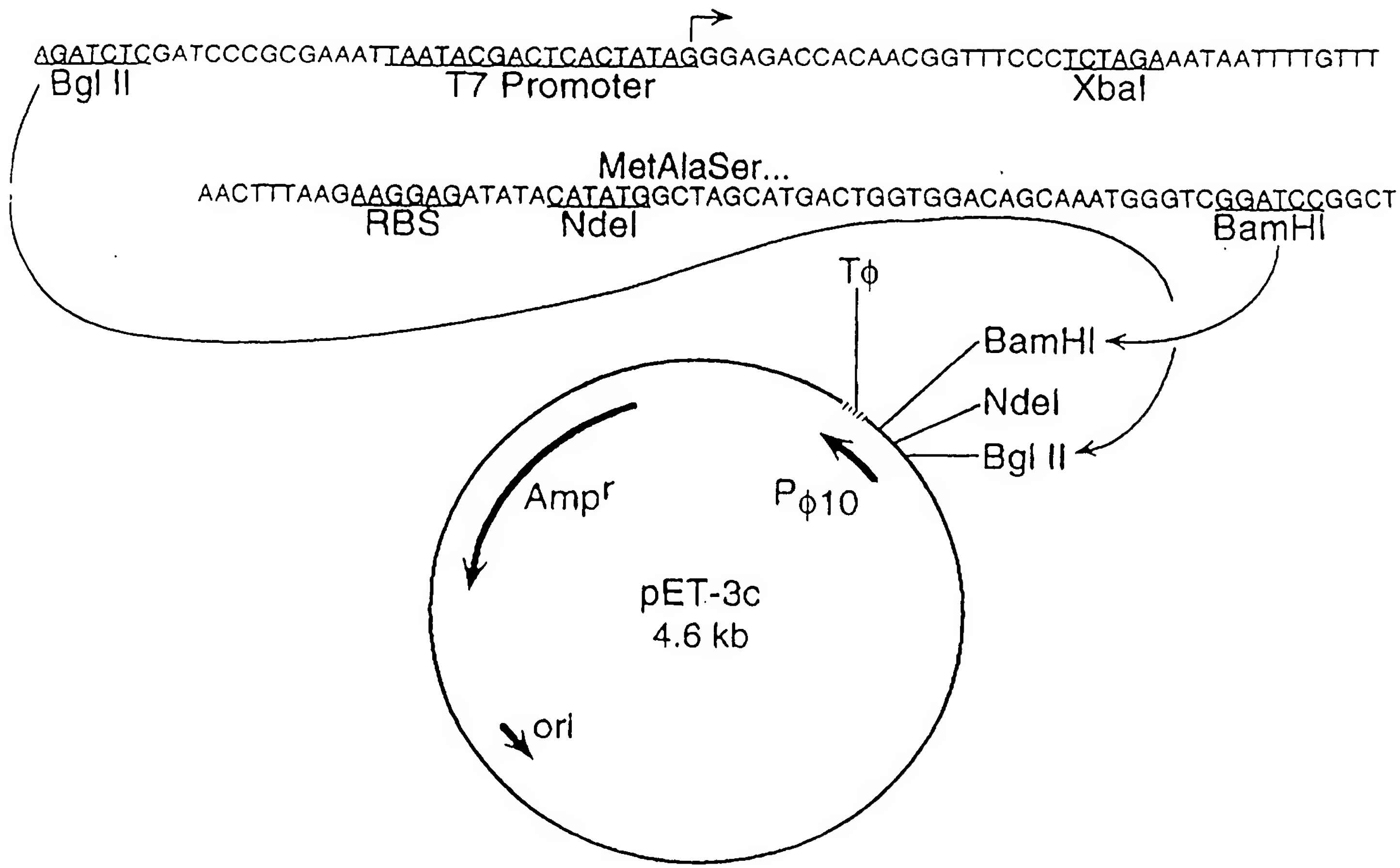
lac IQ: Lac repressor gene

lacZ: Beta-galactosidase alpha fragment

rrnBt: E. coli rrnB transcription terminator

24

FIGURE 15

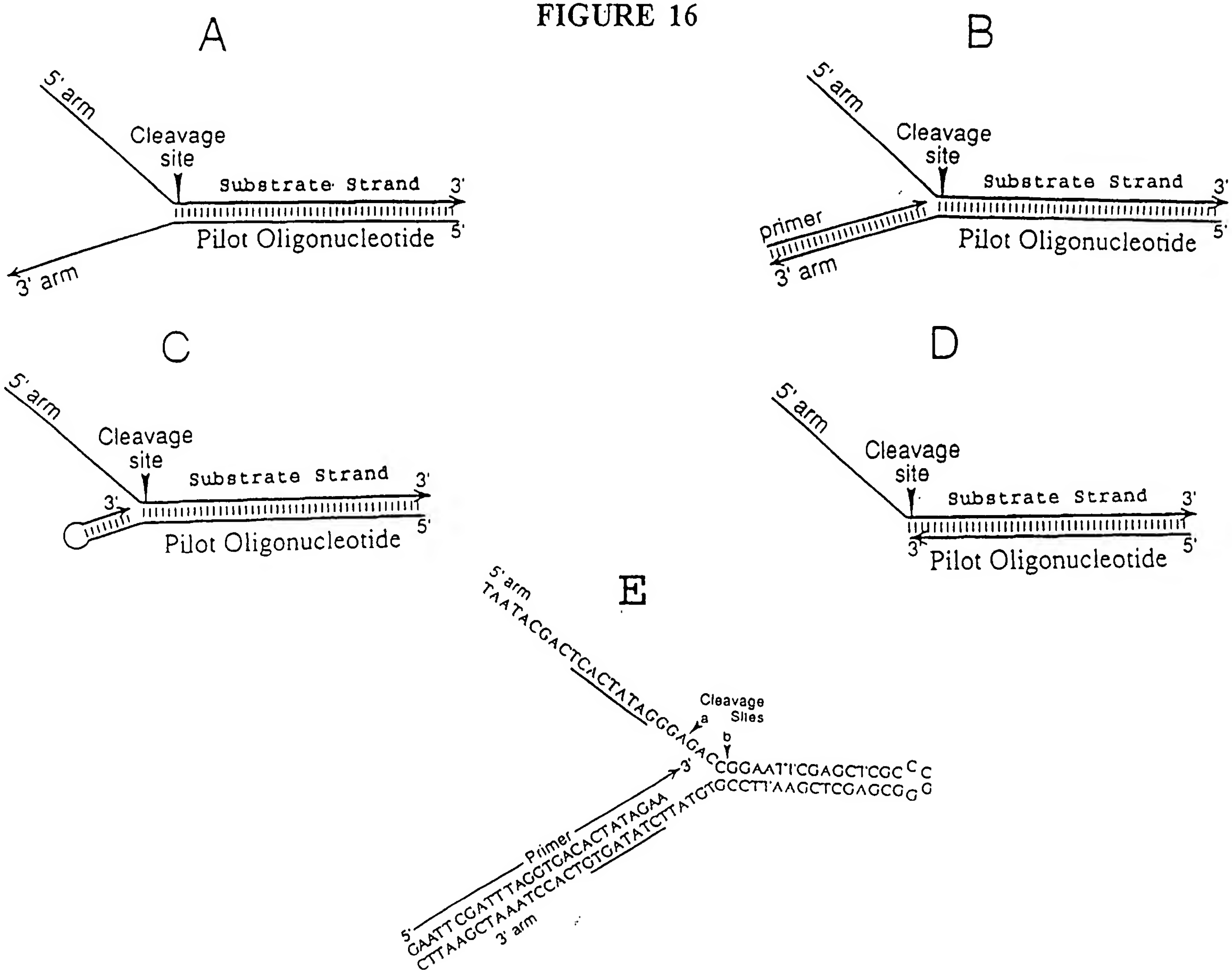


$P_{\phi 10}$: Bacteriophage T7 φ10 promoter

Tφ: T7 φ Terminator

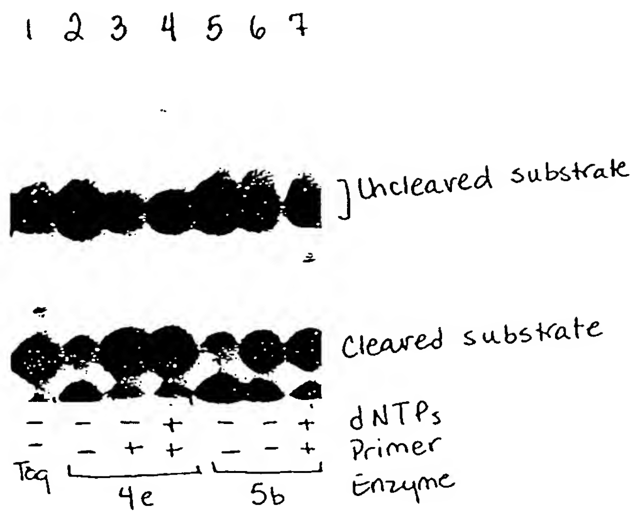
RBS: Ribosome binding site

FIGURE 16


A
B
C
D
E
5' arm
3' arm
Cleavage site
Substrate Strand
Pilot Oligonucleotide
primer
3'
5'
Cleavage Sites
a
b
TAATACGACTCACTATAGGGAGAC
CGGAATTCGAGCTCGCC
GCCTTAAGCTCGAGCGGG
GAATTCGATTTAGGTGACACTATAGAA
CTTAAGCTAAATCCACTGTGATATCTTATGT
Primer

26

FIGURE 17



1 2 3 4 5 6 7
Uncleaved substrate
Cleaved substrate
− − − + − − + dNTPs
− − + + − − + Primer
Taq 4e 5b Enzyme

FIGURE 18

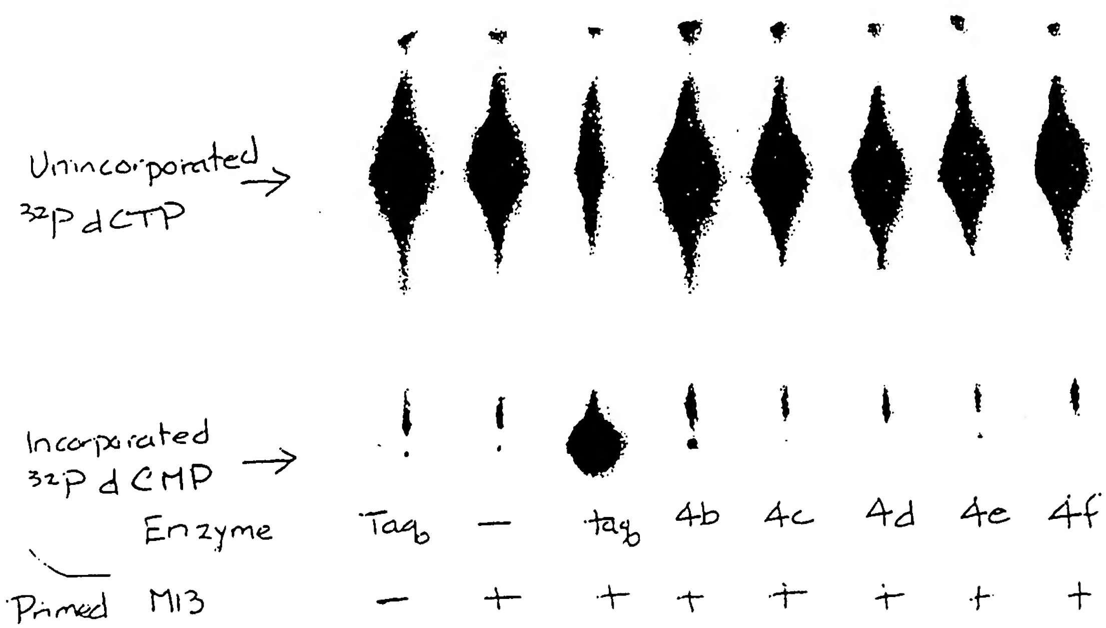
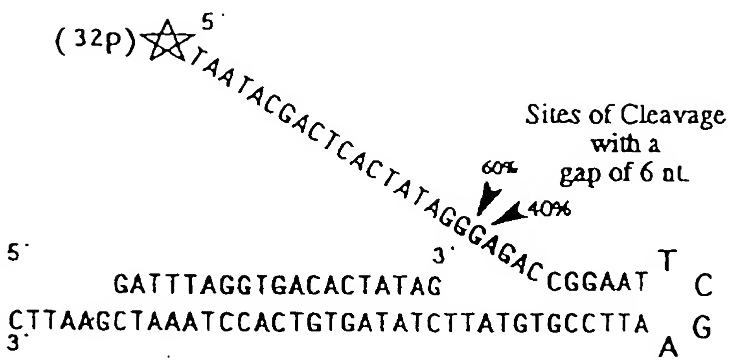

Unincorporated 32P dCTP
Incorporated 32P dCMP
Enzyme
Taq
—
taq
4b
4c
4d
4e
4f
Primed M13
—
+
+
+
+
+
+
+

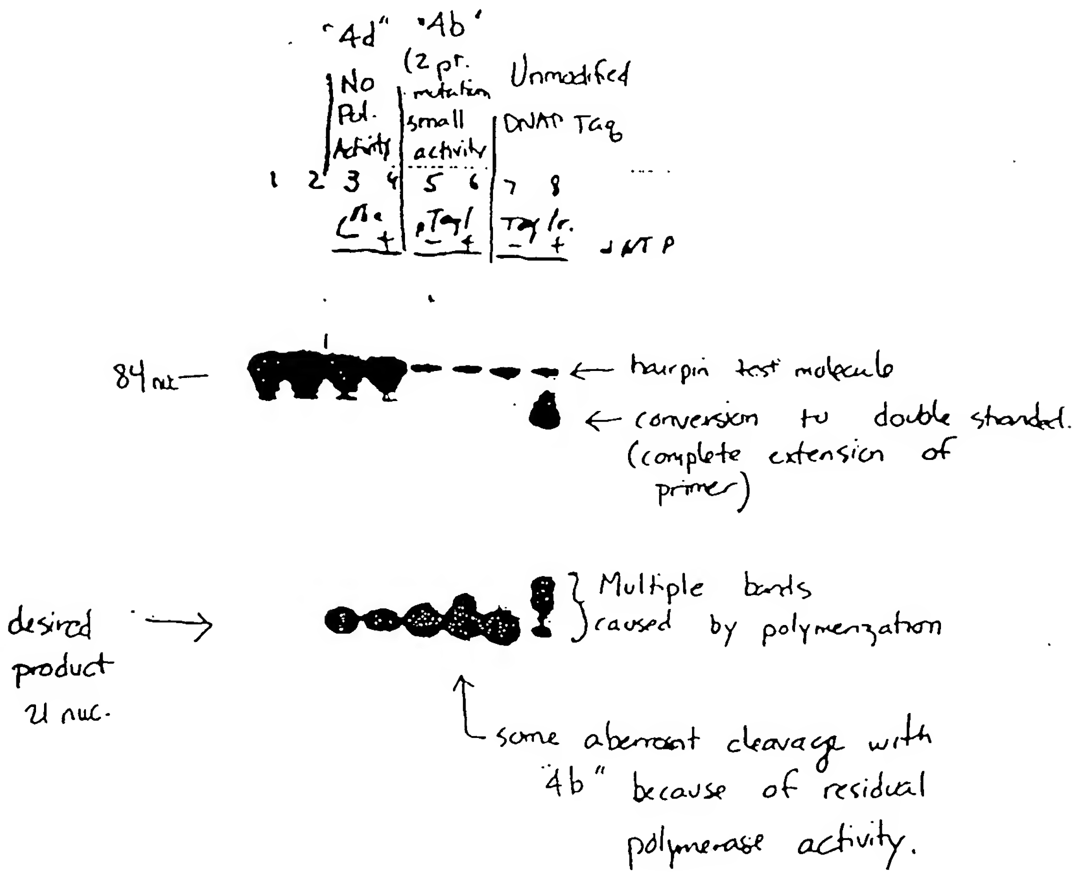
FIGURE 19

A



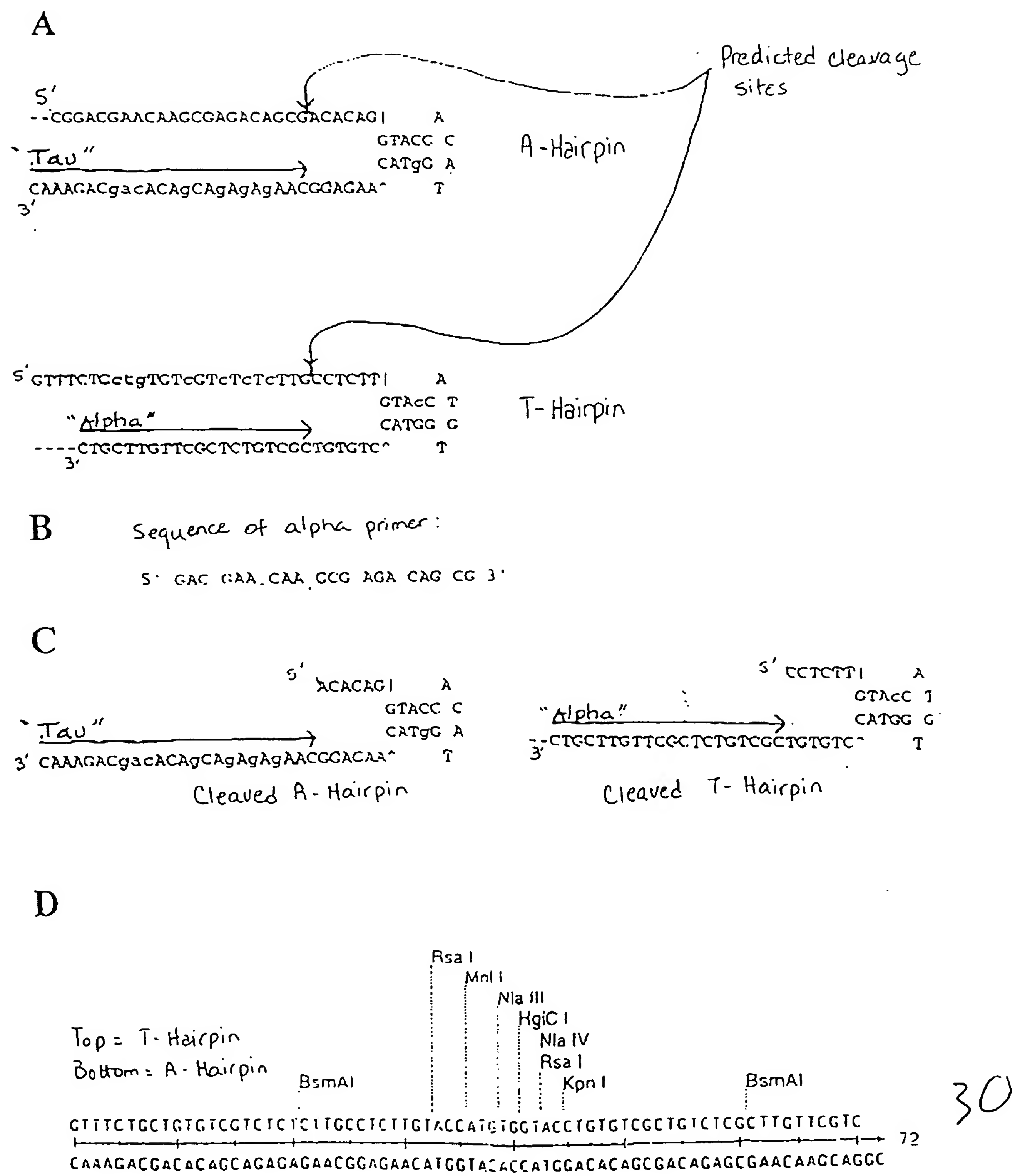
(32P)
5'
TAATACGACTCACTATAGGGAGACCGGAAT
Sites of Cleavage with a gap of 6 nt.
60%
40%
5'
3'
GATTTAGGTGACACTATAG
CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTTA
3'
T
C
G
A

B



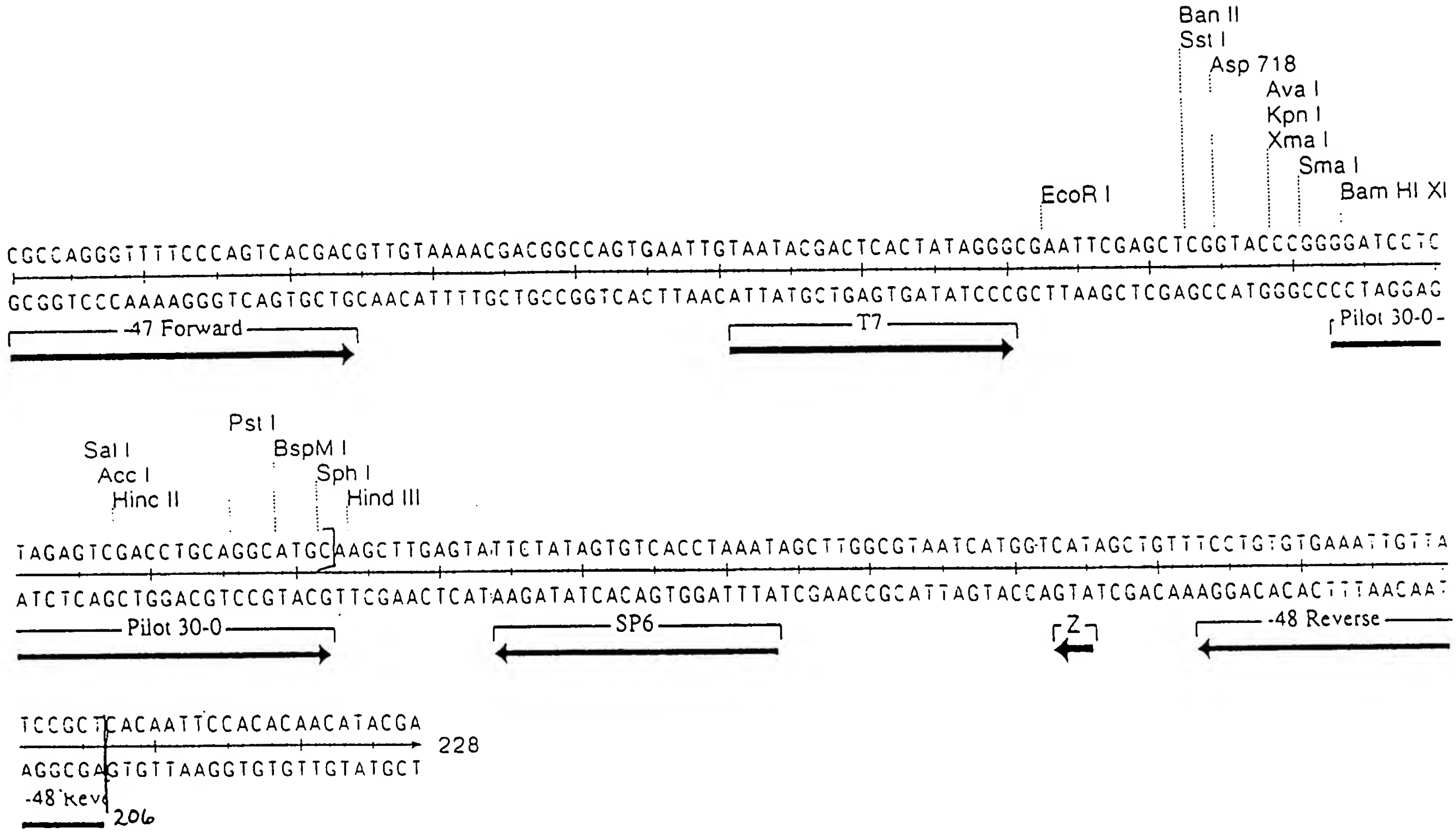
"4d"
No Pol. Activity
"4b"
(2 pt. mutation small activity
Unmodified DNAP Taq
1 2 3 4 5 6 7 8
- + - + - + dNTP
84nt —
← hairpin test molecule
← conversion to double stranded. (complete extension of primer)
Multiple bands caused by polymerization
desired product 21 nuc. →
some aberrant cleavage with "4b" because of residual polymerase activity.

FIGURE 20


A
Predicted cleavage sites
5'
--CGGACGAACAAGCGAGACAGCGACACAGl A
GTACC C
CATgG A
T
"Tau"
CAAAGACgacACAgCAgAgAgAACGGAGAA
3'
A-Hairpin
5' GTTTCTCctgTGTcGTcTcTcTTGCCTCTTl A
GTAcC T
CATGG G
T
"Alpha"
----CTGCTTGTTCGCTCTGTCGCTGTGTC
3'
T-Hairpin
B
Sequence of alpha primer:
5' GAC GAA CAA CCG AGA CAG CG 3'
C
5' ACACAGl A
GTACC C
CATgG A
T
"Tau"
3' CAAAGACgacACAgCAgAgAgAACGGACAA
Cleaved A-Hairpin
5' CCTCTTl A
GTAcC T
CATGG G
T
"Alpha"
--CTGCTTGTTCGCTCTGTCGCTGTGTC
3'
Cleaved T-Hairpin
D
Top = T-Hairpin
Bottom = A-Hairpin
BsmAI
Rsa I
Mnl I
Nla III
HgiC I
Nla IV
Rsa I
Kpn I
BsmAI
72

30

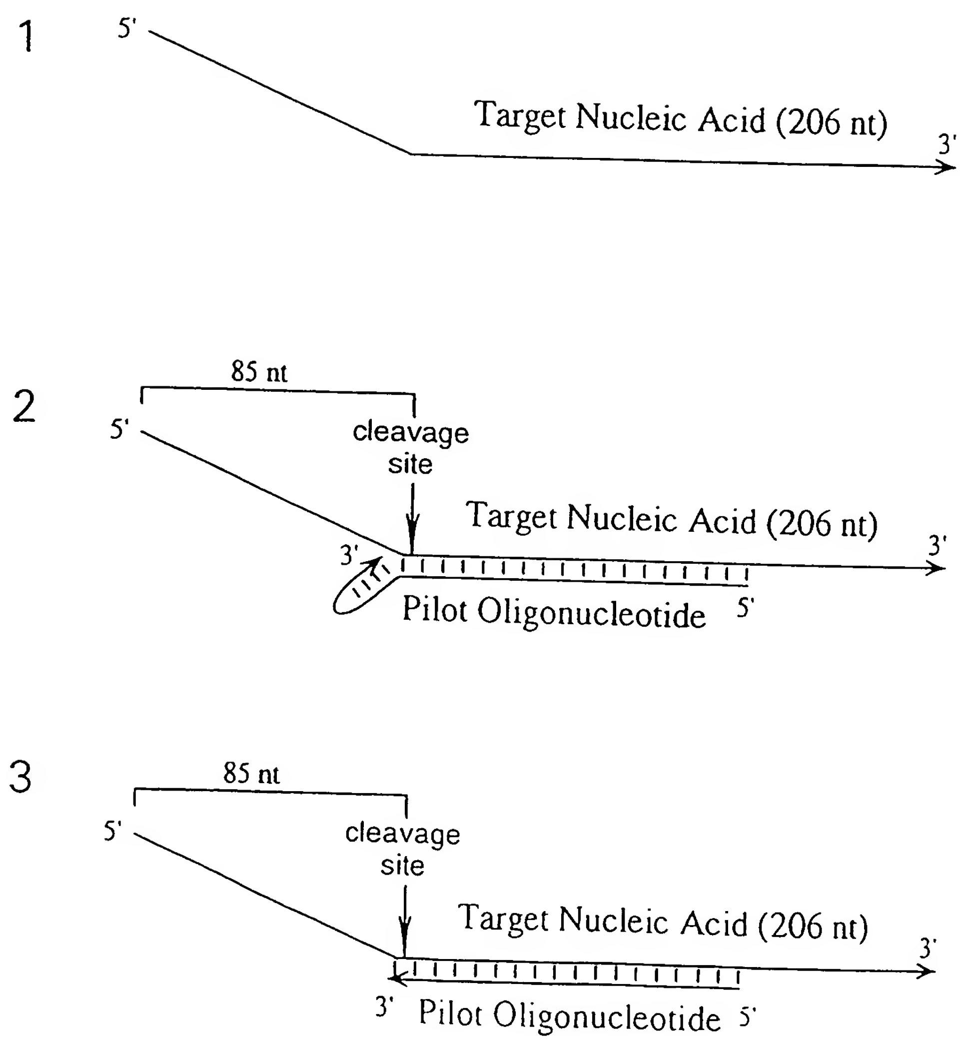
FIGURE 21



Ban II
Sst I
Asp 718
Ava I
Kpn I
Xma I
Sma I
Bam HI XI
EcoR I
CGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGGGATCCTC
GCGGTCCCAAAAGGGTCAGTGCTGCAACATTTTGCTGCCGGTCACTTAACATTATGCTGAGTGATATCCCGCTTAAGCTCGAGCCATGGGCCCCTAGGAG
-47 Forward
T7
Pilot 30-0-
Pst I
Sal I
BspM I
Acc I
Sph I
Hinc II
Hind III
TAGAGTCGACCTGCAGGCATGCAAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTA
ATCTCAGCTGGACGTCCGTACGTTCGAACTCATAAGATATCACAGTGGATTTATCGAACCGCATTAGTACCAGTATCGACAAAGGACACACTTTAACAAT
Pilot 30-0
SP6
Z
-48 Reverse
TCCGCTCACAATTCCACACAACATACGA
228
AGGCGAGTGTTAAGGTGTGTTGTATGCT
-48 Reve
206

31

FIGURE 22A



1
5'
Target Nucleic Acid (206 nt)
3'
2
85 nt
5'
cleavage site
Target Nucleic Acid (206 nt)
3'
3'
Pilot Oligonucleotide
5'
3
85 nt
5'
cleavage site
Target Nucleic Acid (206 nt)
3'
3'
Pilot Oligonucleotide
5'

FIGURE 22B

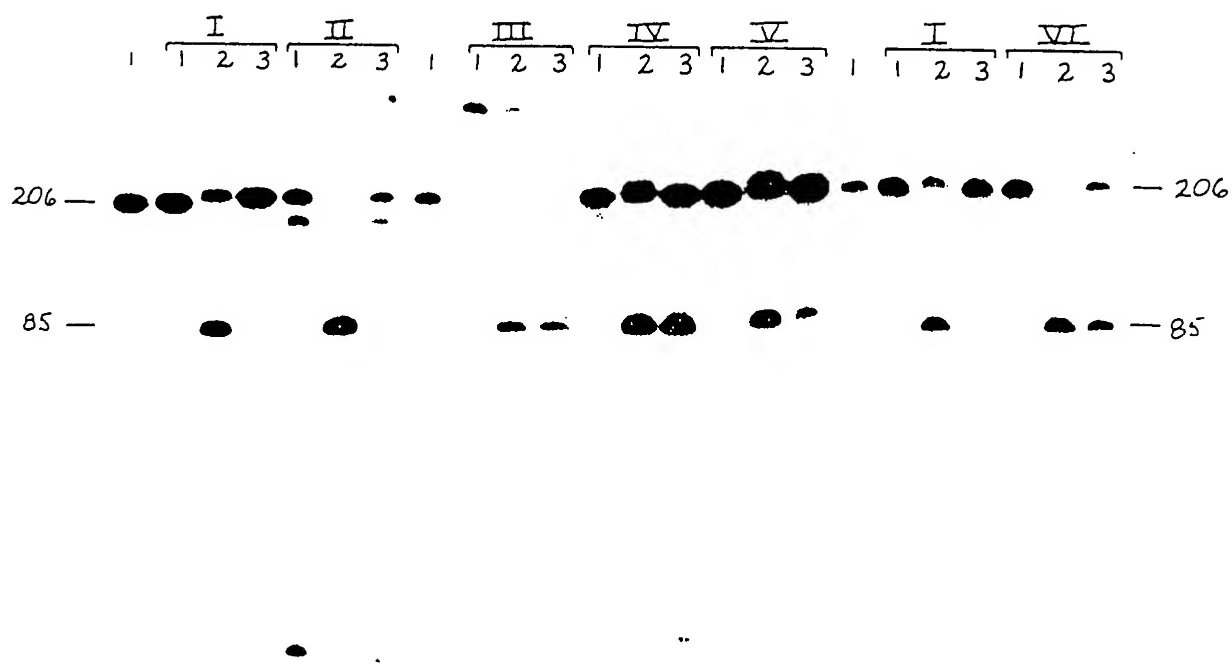


FIGURE 23

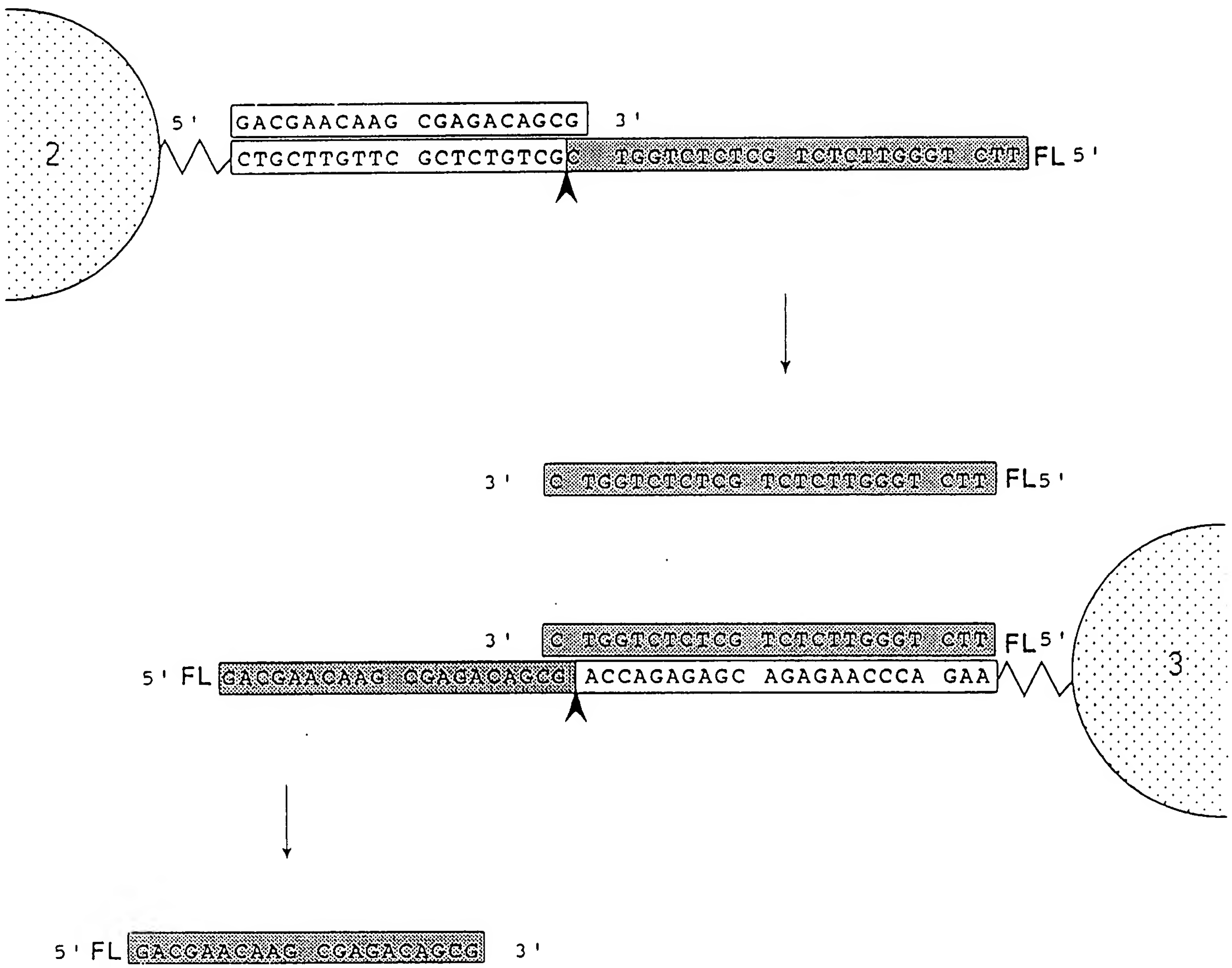

5'
GACGAACAAG CGAGACAGCG
3'
2
CTGCTTGTTC GCTCTGTCGC TGGTCTCTCG TCTCTTGGGT CTT
FL 5'
3'
C TGGTCTCTCG TCTCTTGGGT CTT
FL5'
3'
C TGGTCTCTCG TCTCTTGGGT CTT
FL5'
5' FL
GACGAACAAG CGAGACAGCG ACCAGAGAGC AGAGAACCCA GAA
3
5' FL
GACGAACAAG CGAGACAGCG
3'

FIGURE 24

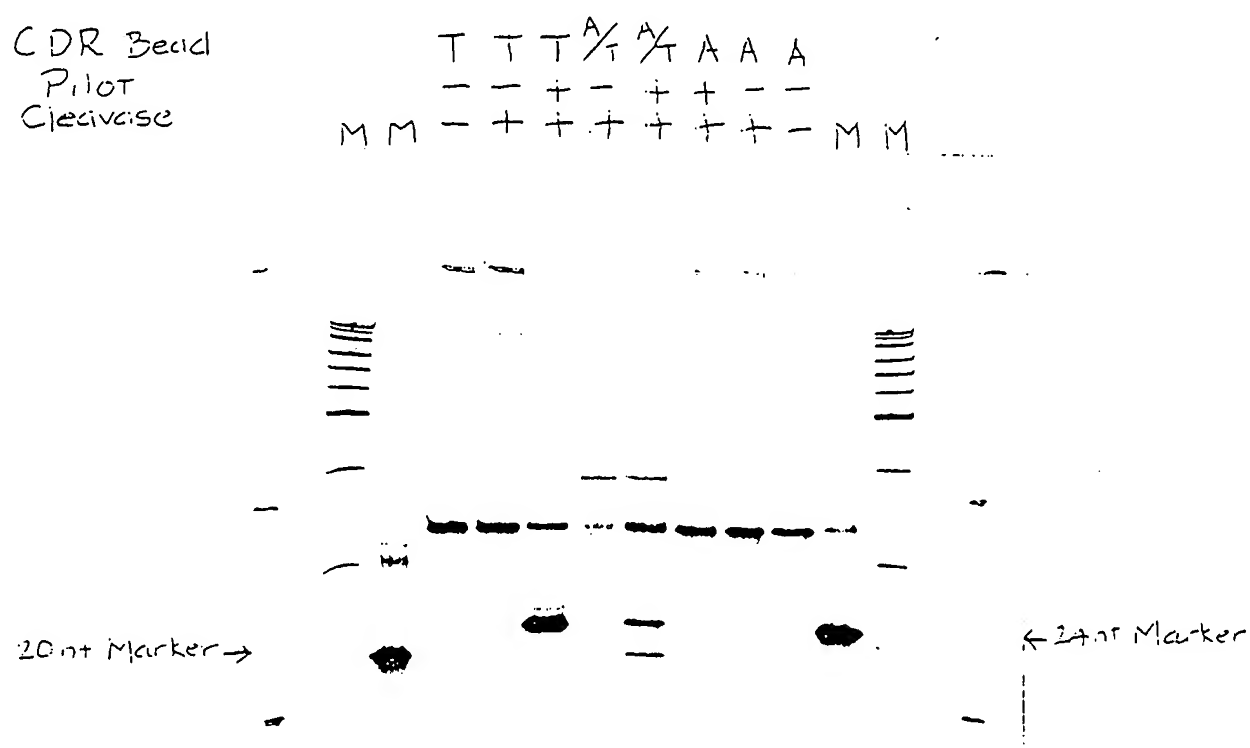

CDR Bead
Pilot
Cleavase
T T T A/T A/T A A A
— — + — + + — —
M M — + + + + + + — M M
20nt Marker →
← 24nt Marker

FIGURE 25

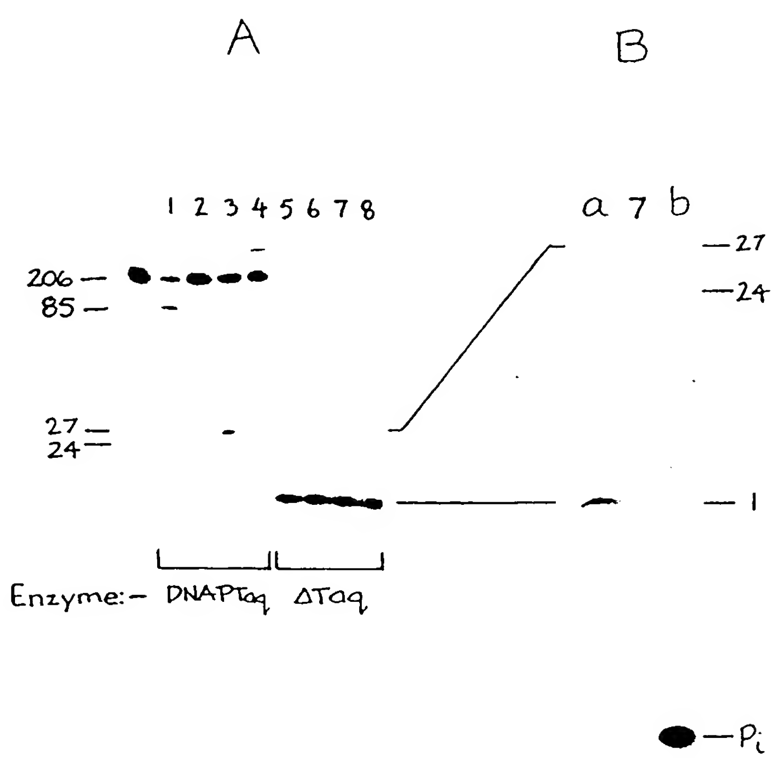
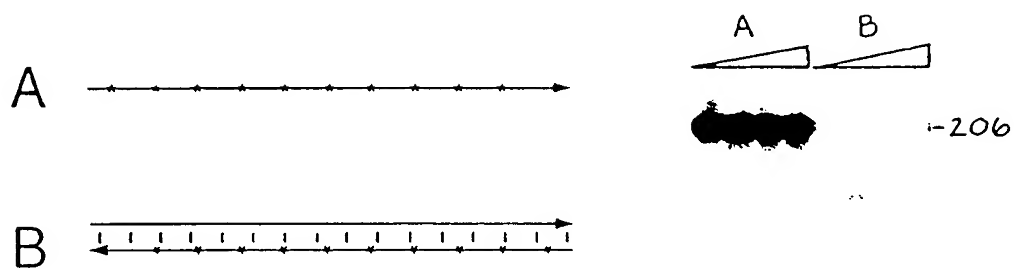
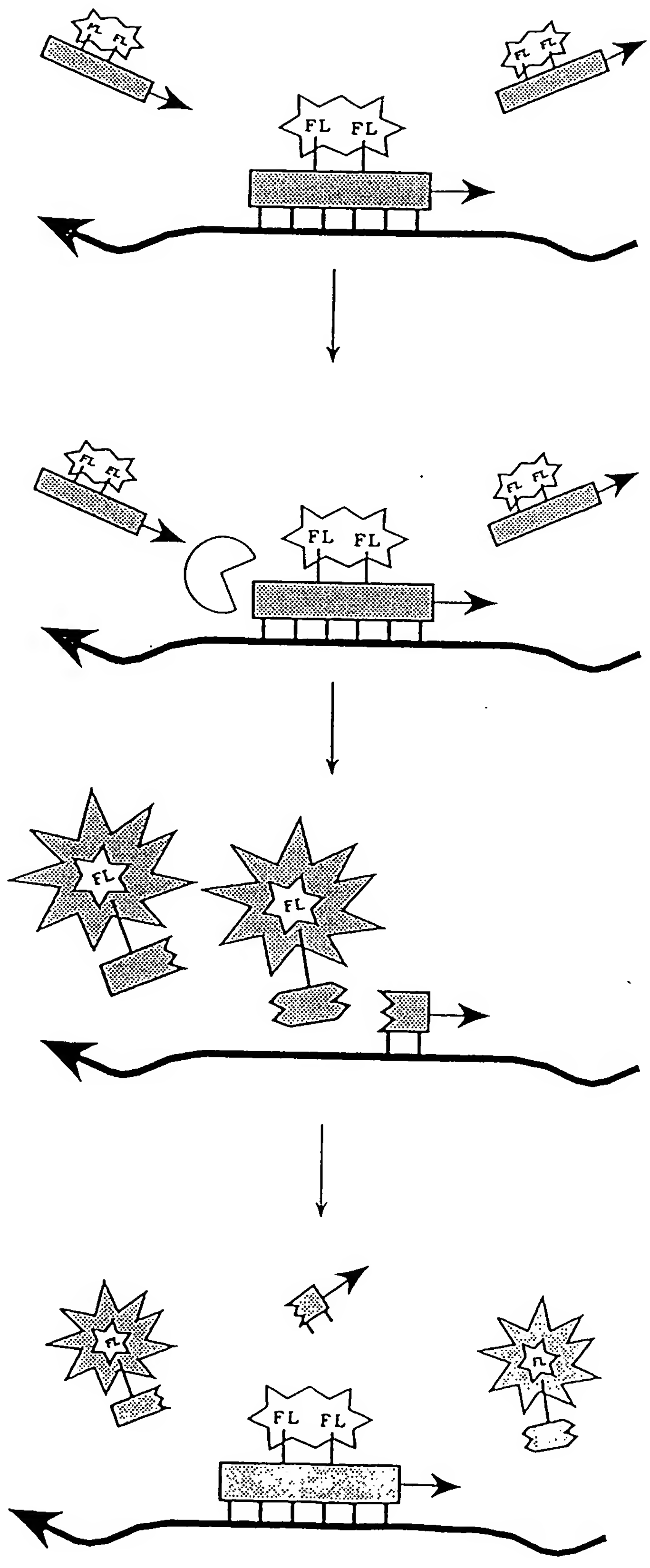


FIGURE 26



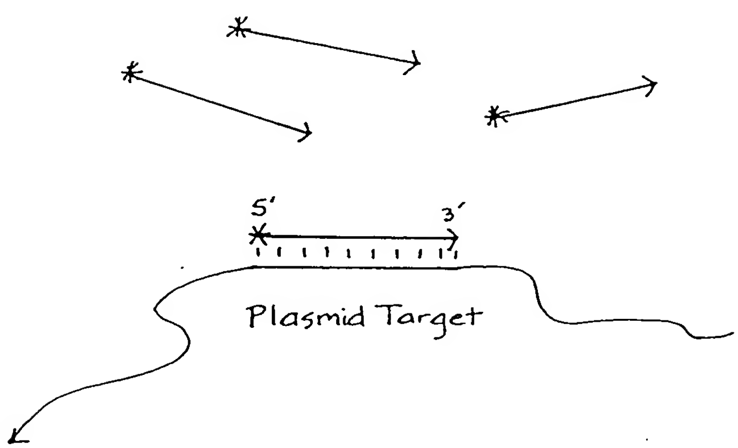
* = ^{32}P

FIGURE 27



10074328.021202

FIGURE 28A



* = ^{32}P 5' terminal phosphate

FIGURE 28B

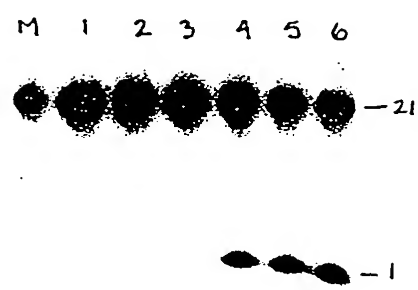


FIGURE 29

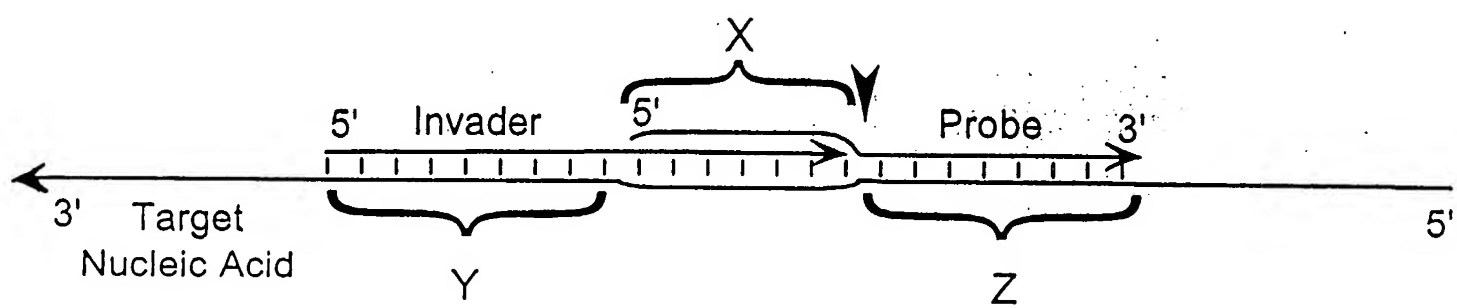
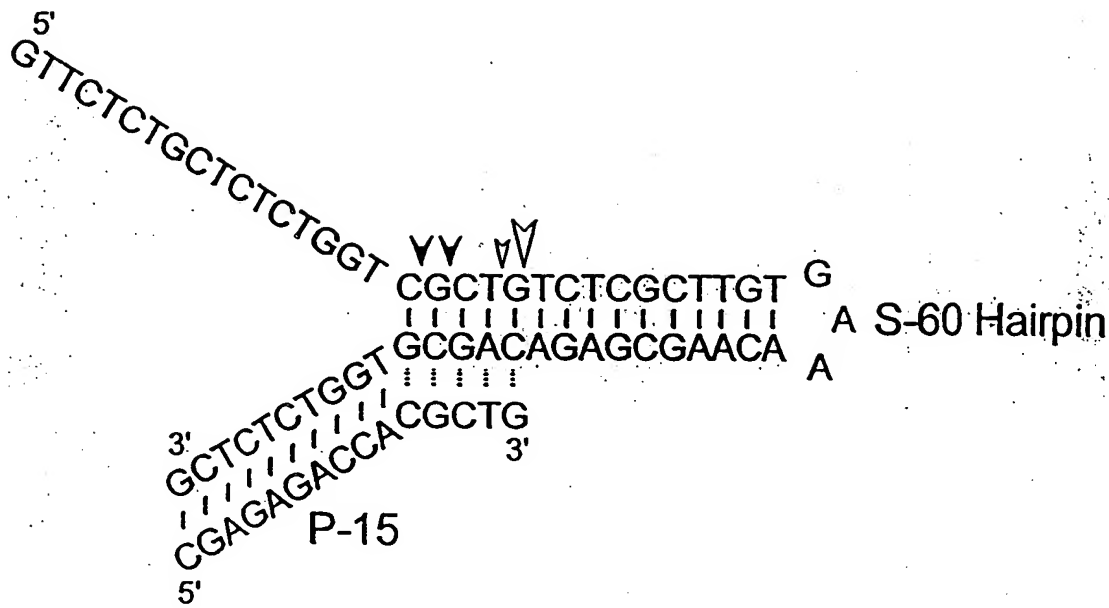


FIGURE 30



10074320 .022102

FIGURE 31

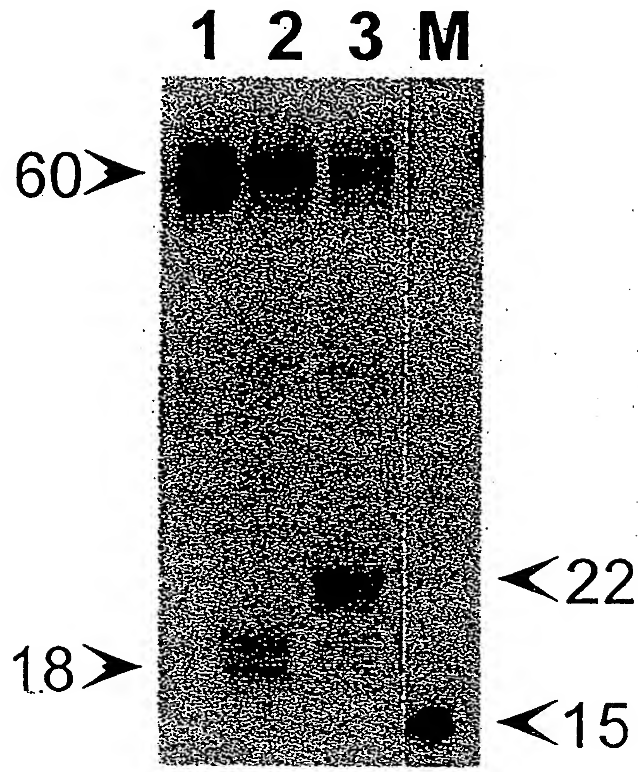


FIGURE 32

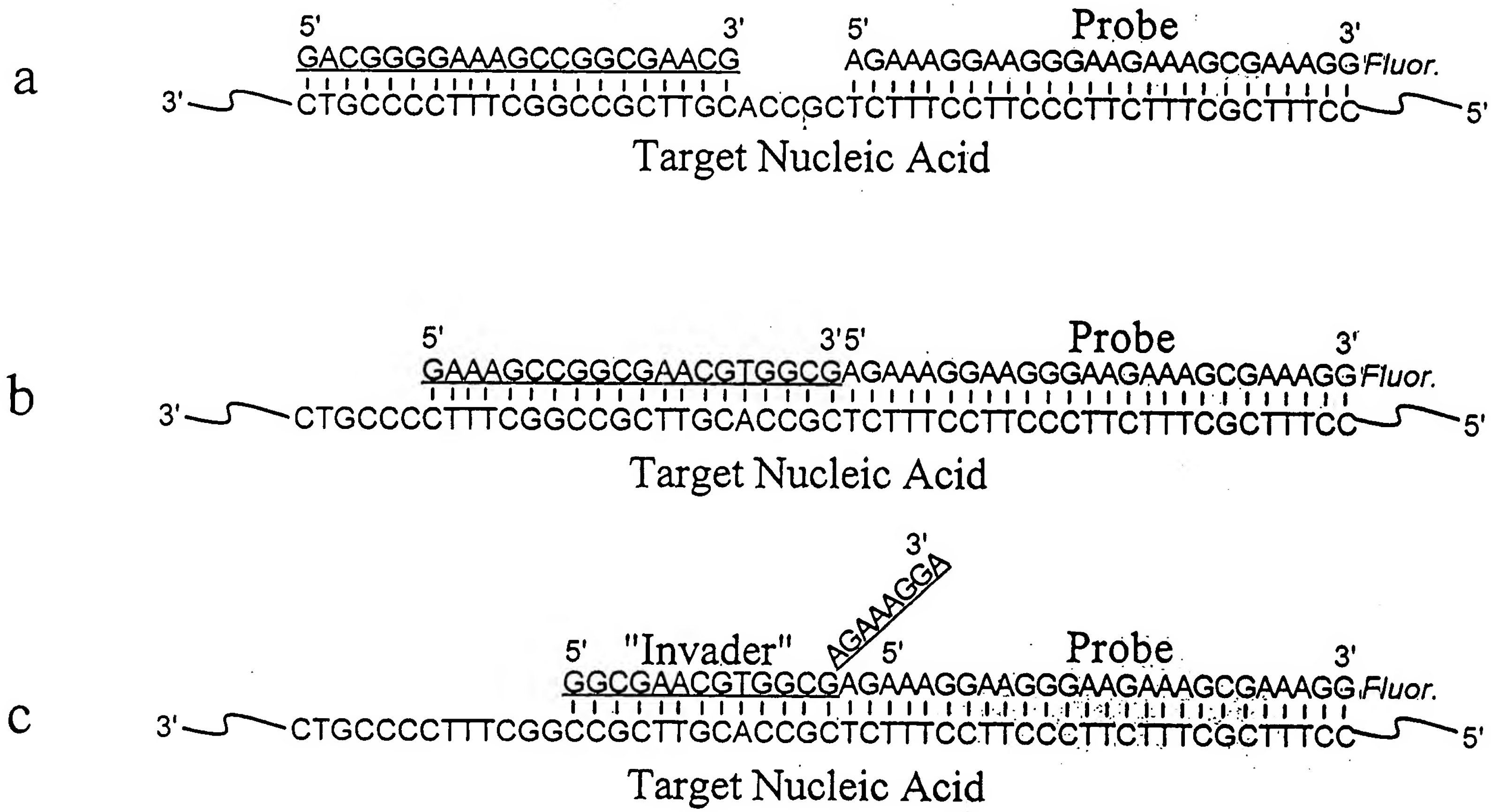

a
5' GACGGGGAAAGCCGGCGAACG 3'
5' AGAAAGGAAGGAAGAAAGCGAAAGG 3' Fluor.
Probe
3' CTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'
Target Nucleic Acid
b
5' GAAAGCCGGCGAACGTGGCG 3'
5' AGAAAGGAAGGAAGAAAGCGAAAGG 3' Fluor.
Probe
3' CTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'
Target Nucleic Acid
c
5' GGCGAACGTGGCG
"Invader"
AGAAAGGA 3'
5' AGAAAGGAAGGAAGAAAGCGAAAGG 3' Fluor.
Probe
3' CTGCCCCTTTCGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'
Target Nucleic Acid

FIGURE 35

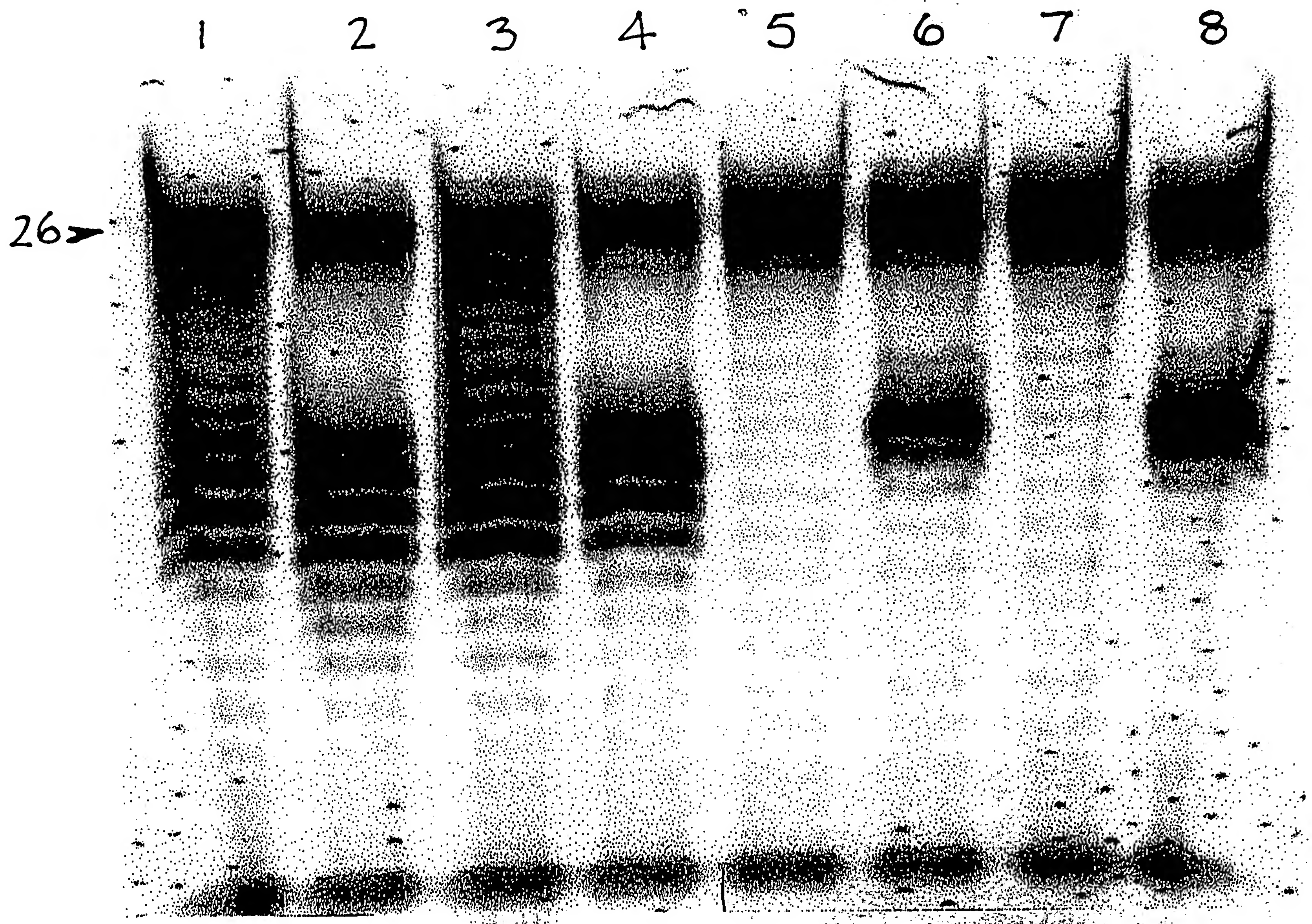


FIGURE 34

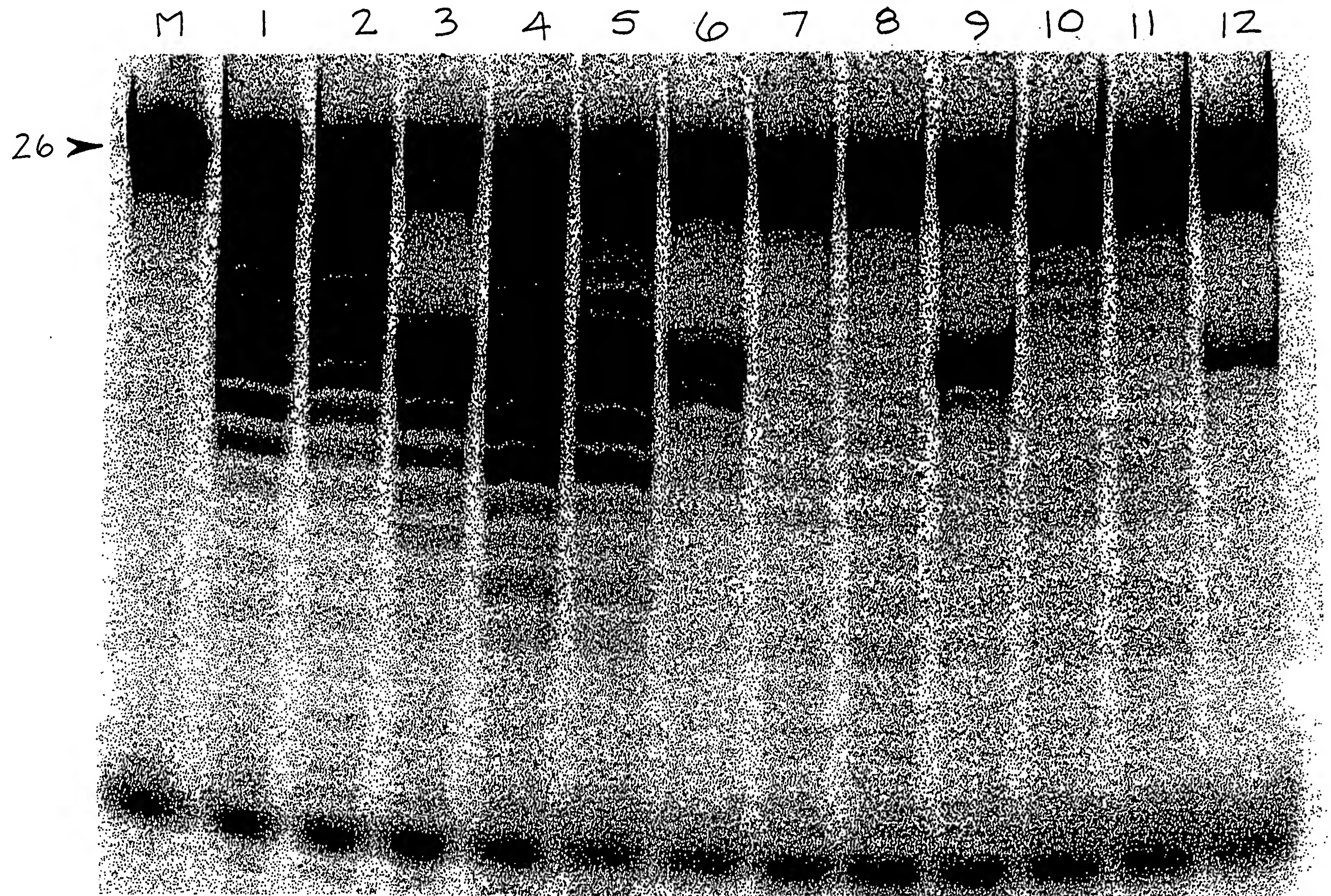


FIGURE 35

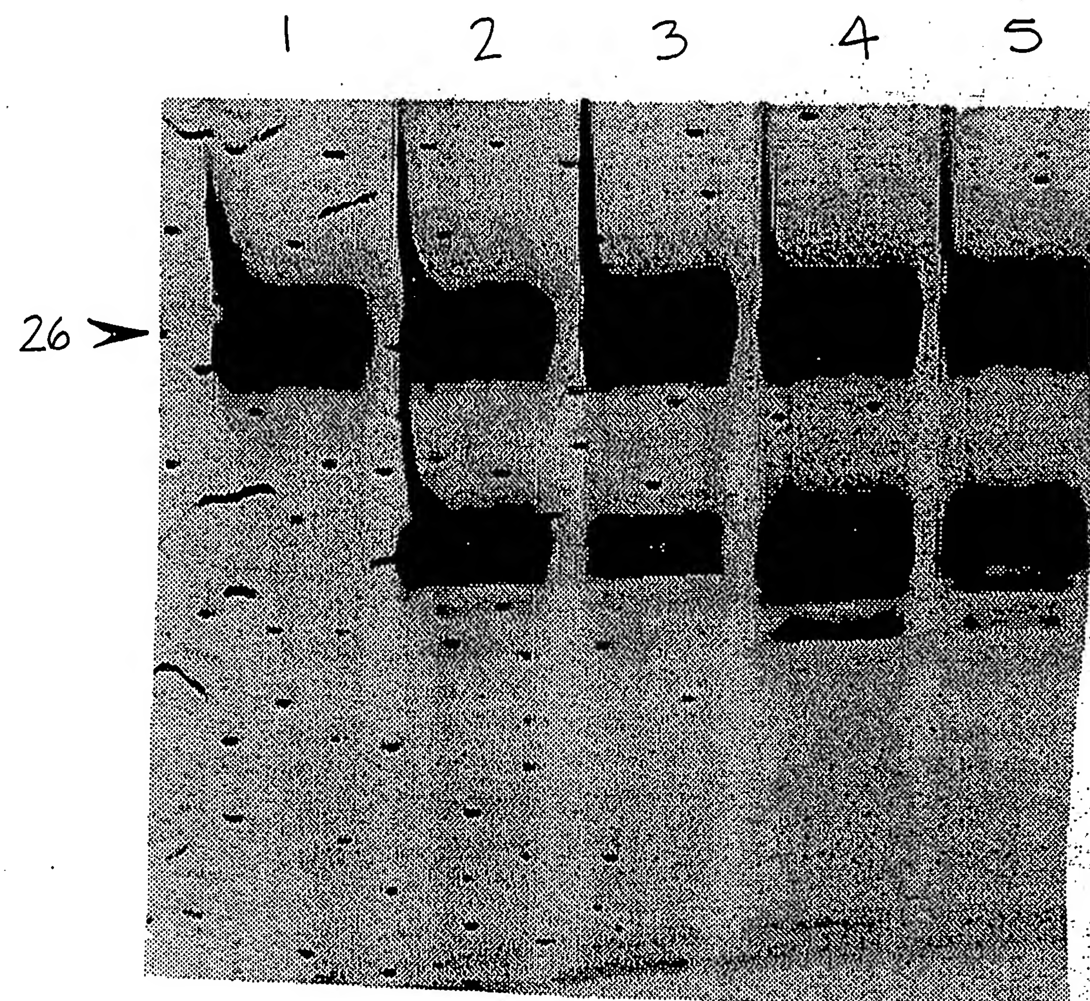
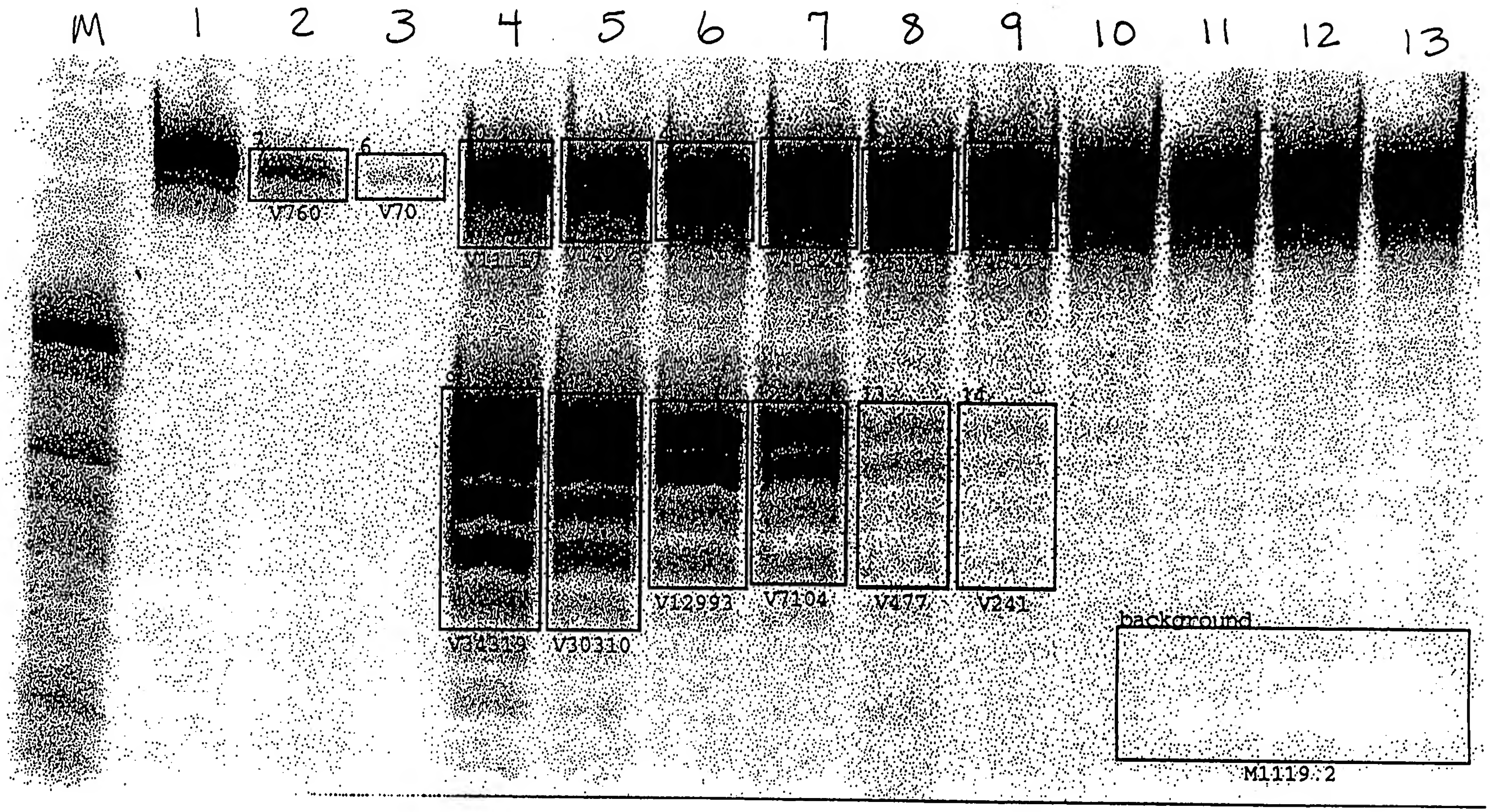


FIGURE 36



M
1
2
3
4
5
6
7
8
9
10
11
12
13
V760
V70
V30310
V12993
V7104
V477
V241
background
M1119.2

FIGURE 37

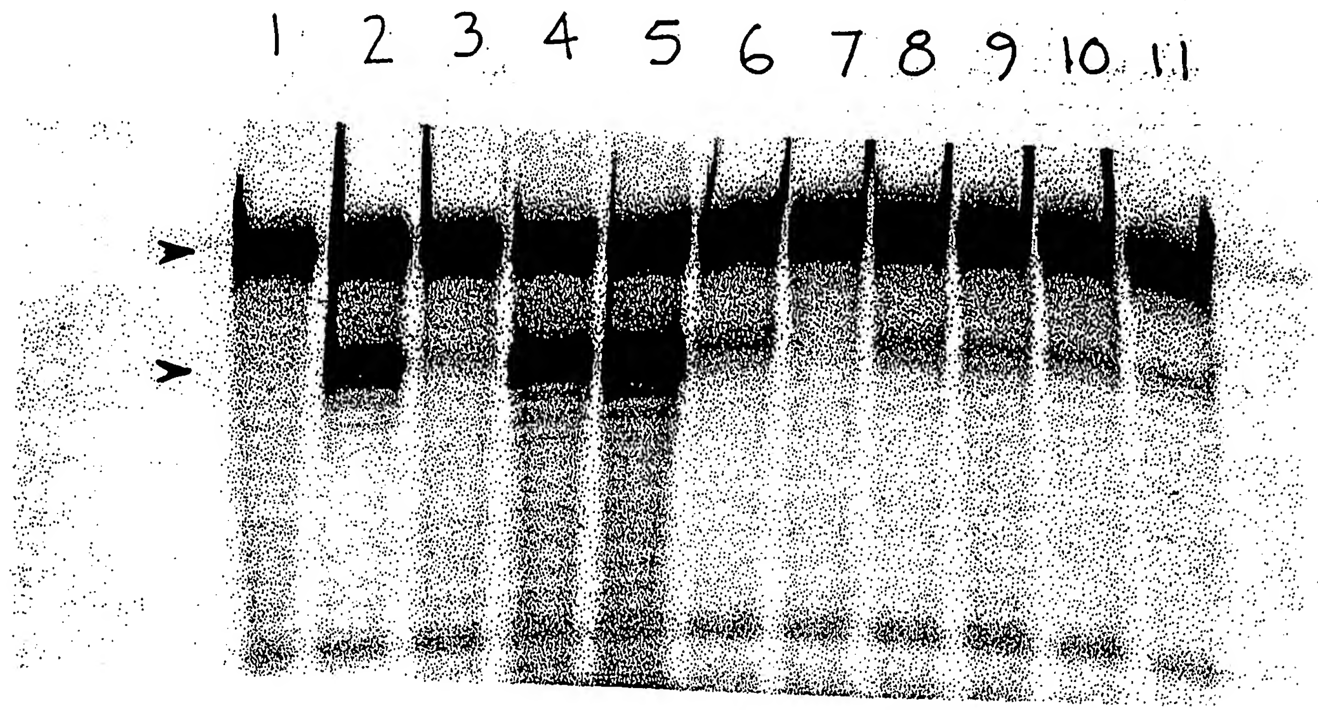


FIGURE 38

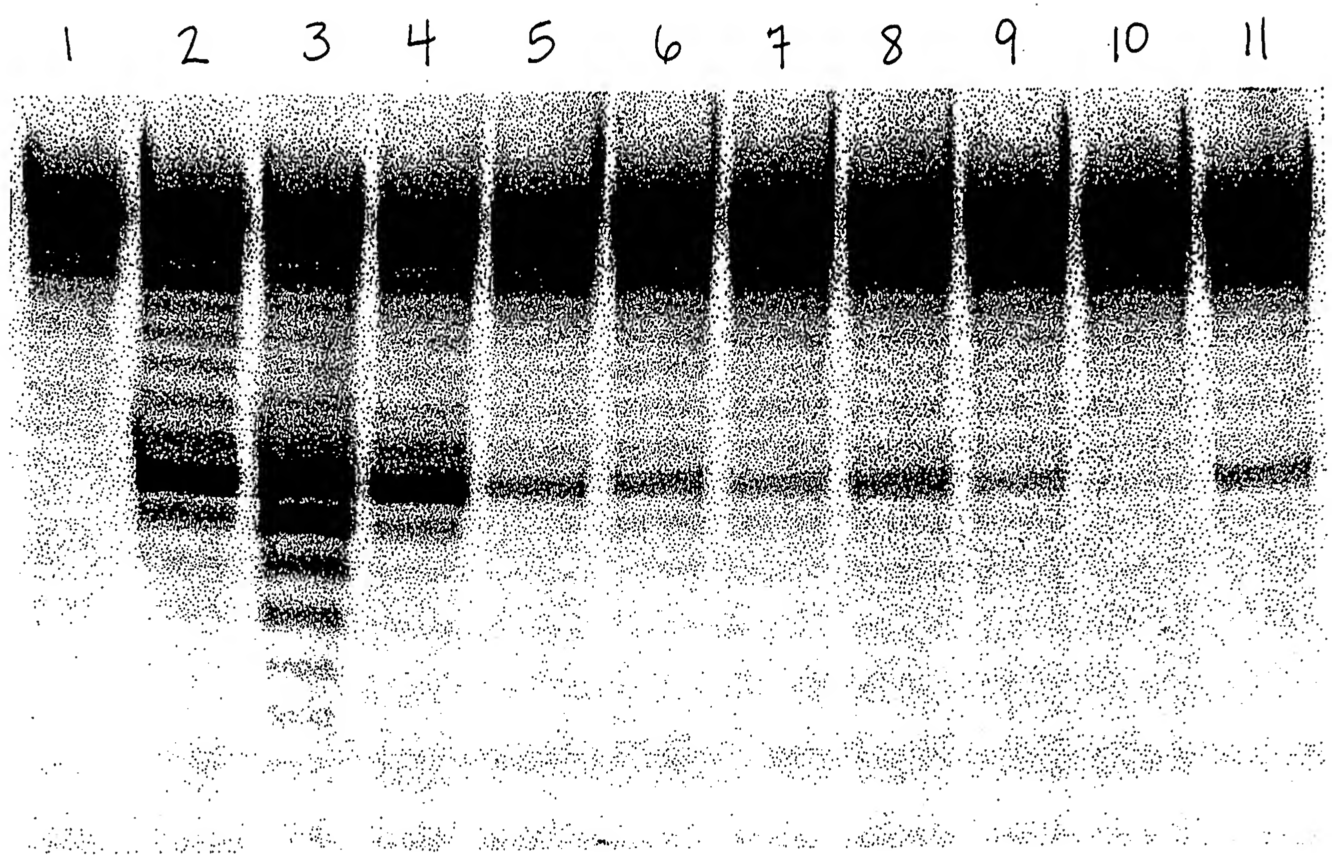


FIGURE 39

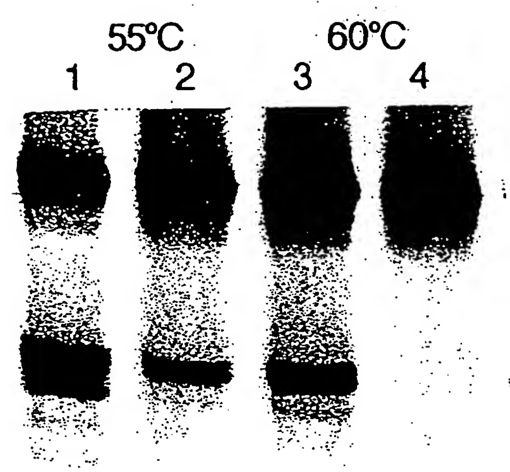


FIGURE 40

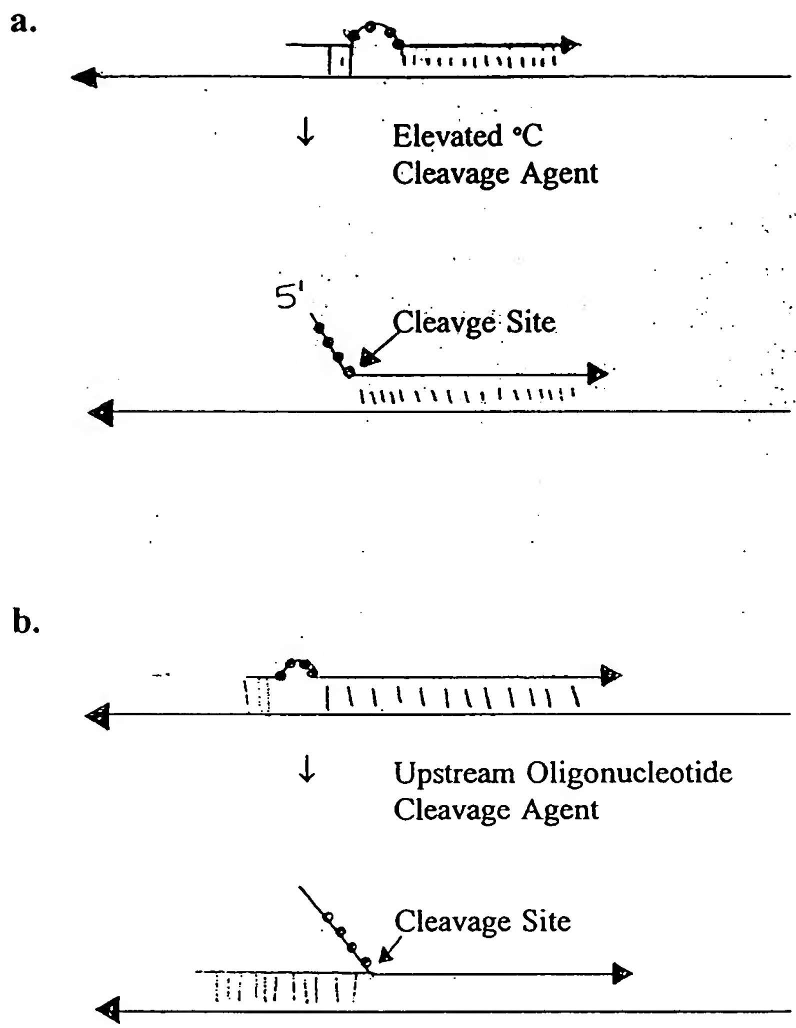

a.
Elevated °C
Cleavage Agent
5'
Cleavge Site
b.
Upstream Oligonucleotide
Cleavage Agent
Cleavage Site

FIGURE 41

```
Invasive Cleavage Directing Oligo                  cleavage site
                                                        ↓
                            5'-cy-3agaaaggA agggaagaaagcgaaaggt-3' ← Probe oligo
     5'-gccggcgaacgtggcgagaaagga
3'-ctgcccctttcggccgcttgcaccgctctttccttcccttctttcgctttcc-5'
```

FIGURE 42

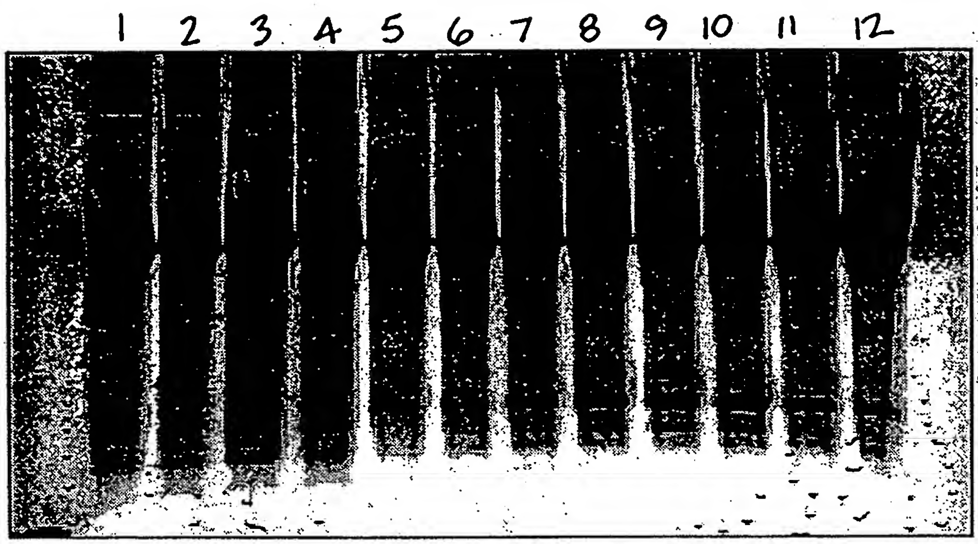


FIGURE 43

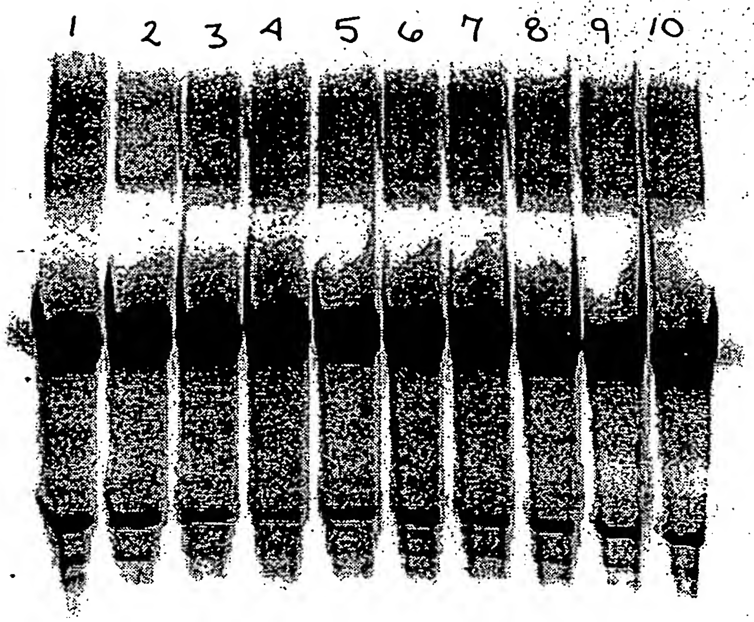


FIGURE 44

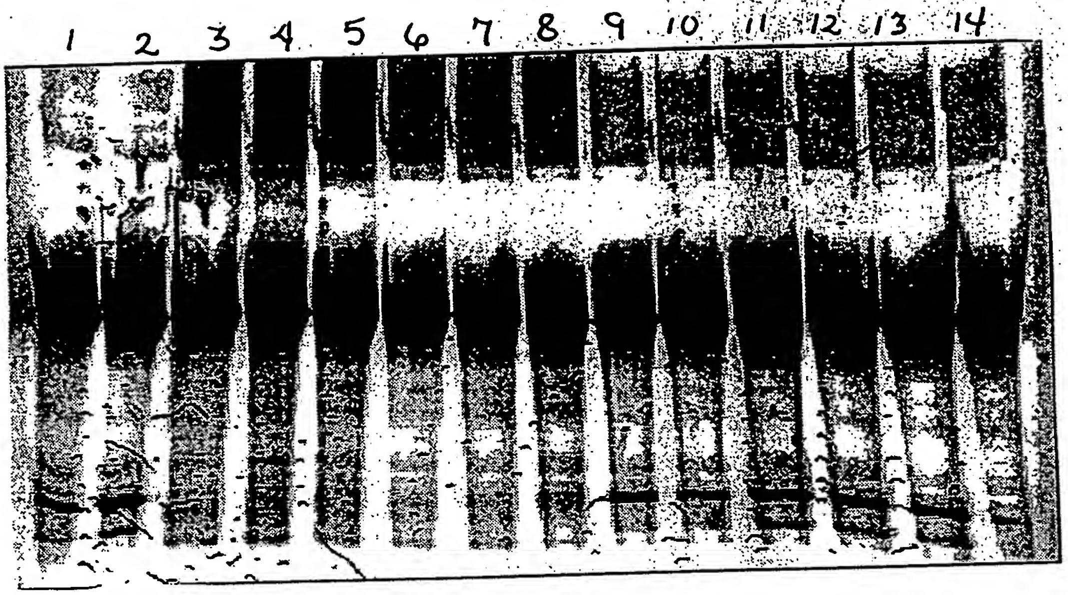


FIGURE 45

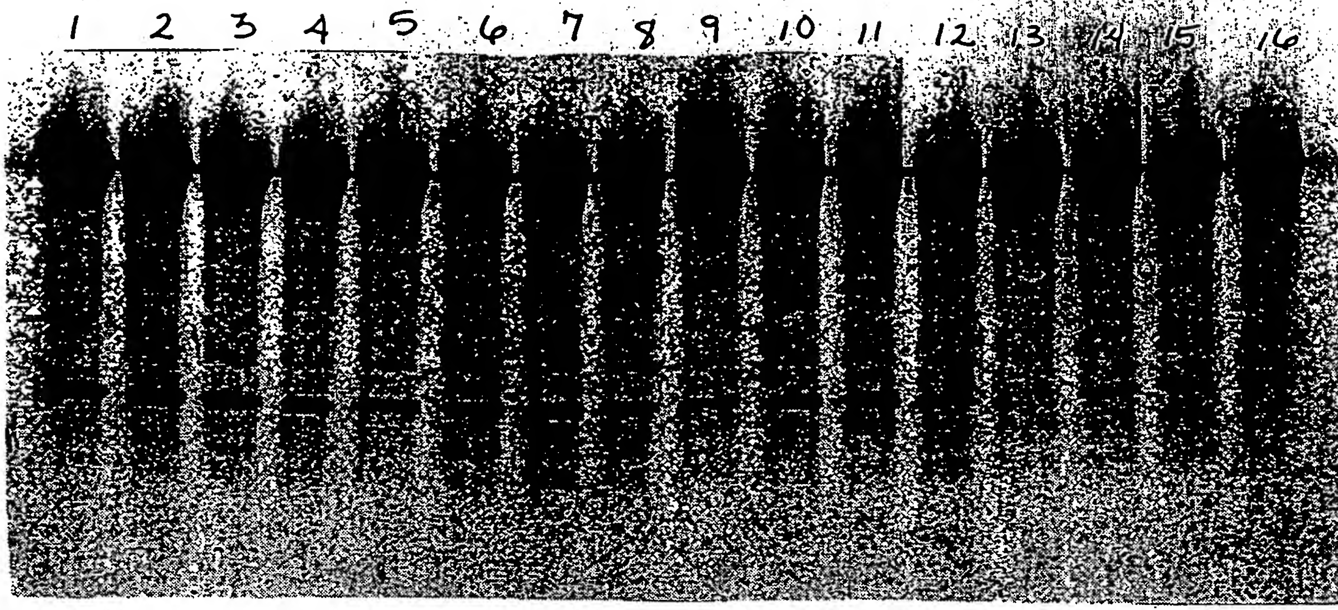


FIGURE 46

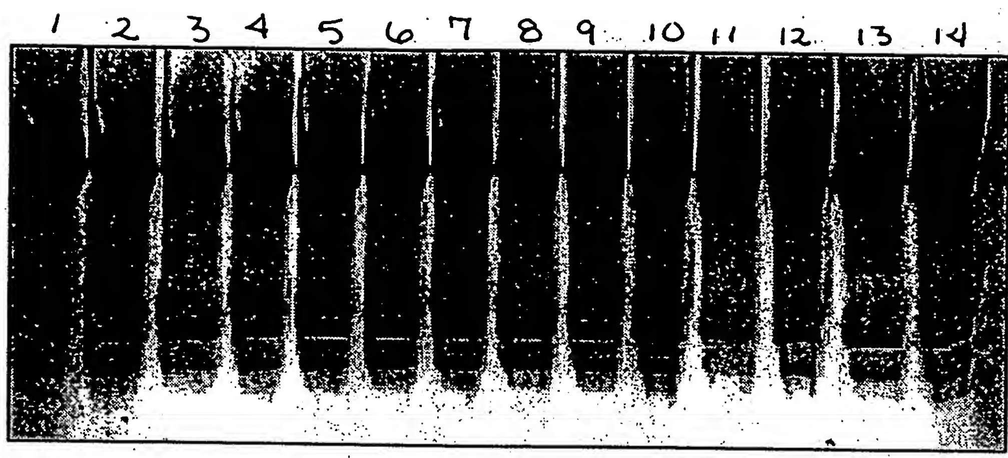
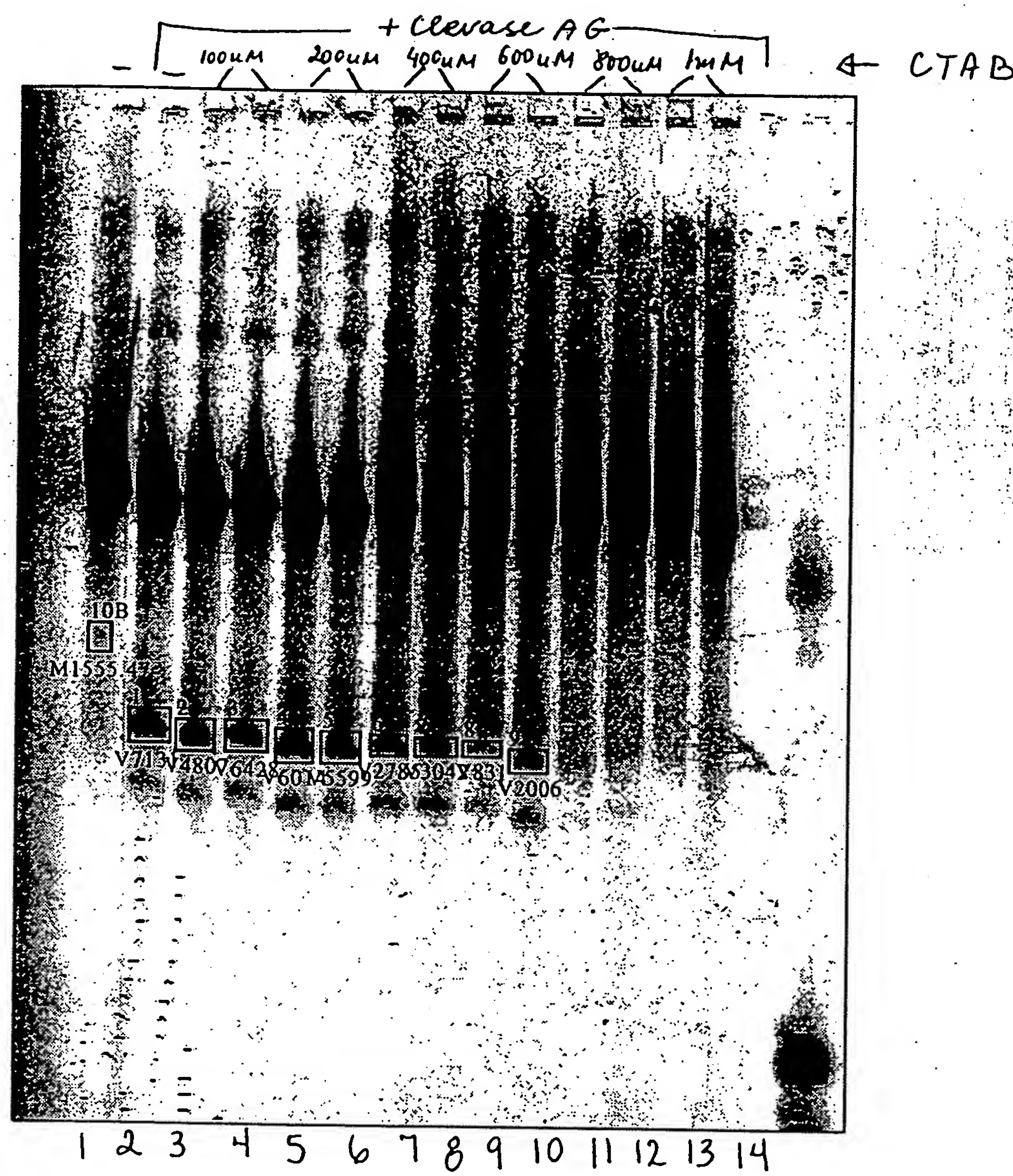


FIGURE 47



+ Clevase AG
100uM
200uM
400uM
600uM
800uM
1mM
CTAB
10B
M1555
V713
V480
V642
V2006
1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIGURE 48

FIGURE 49

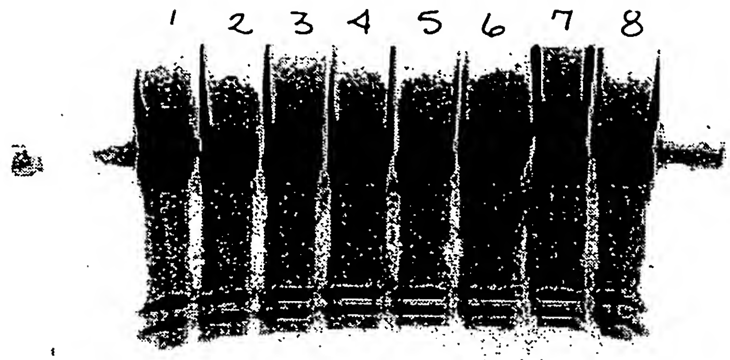


FIGURE 50

FIGURE 51

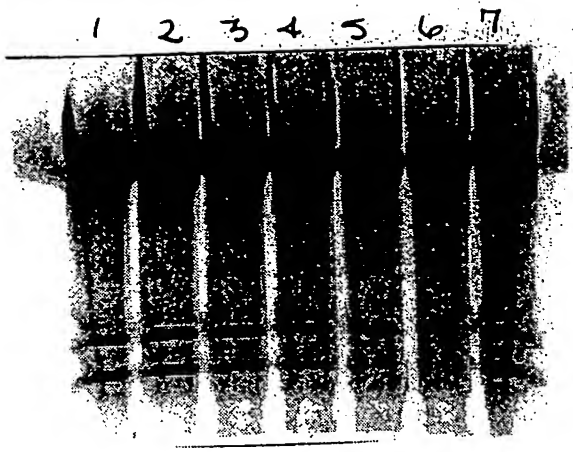
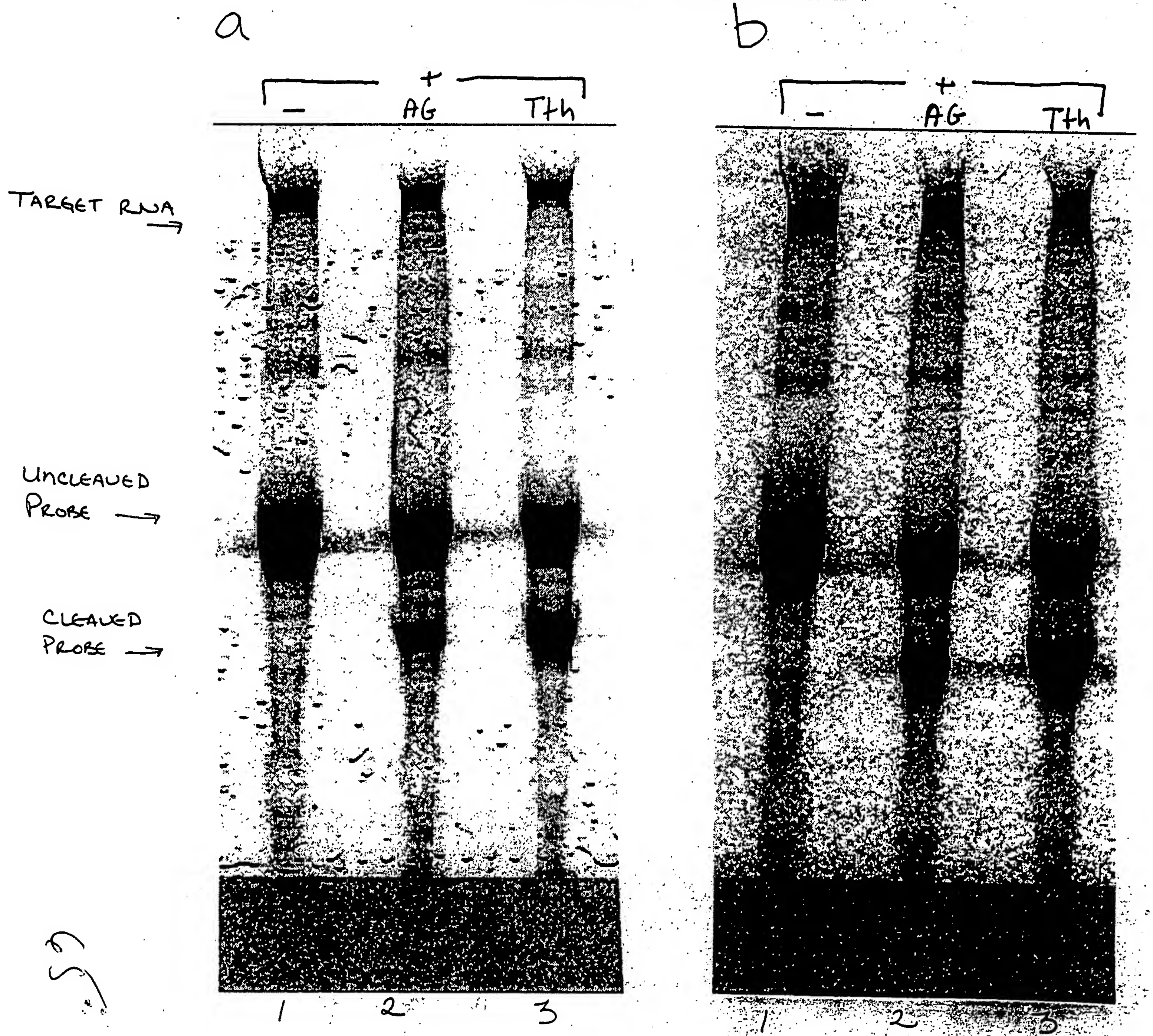


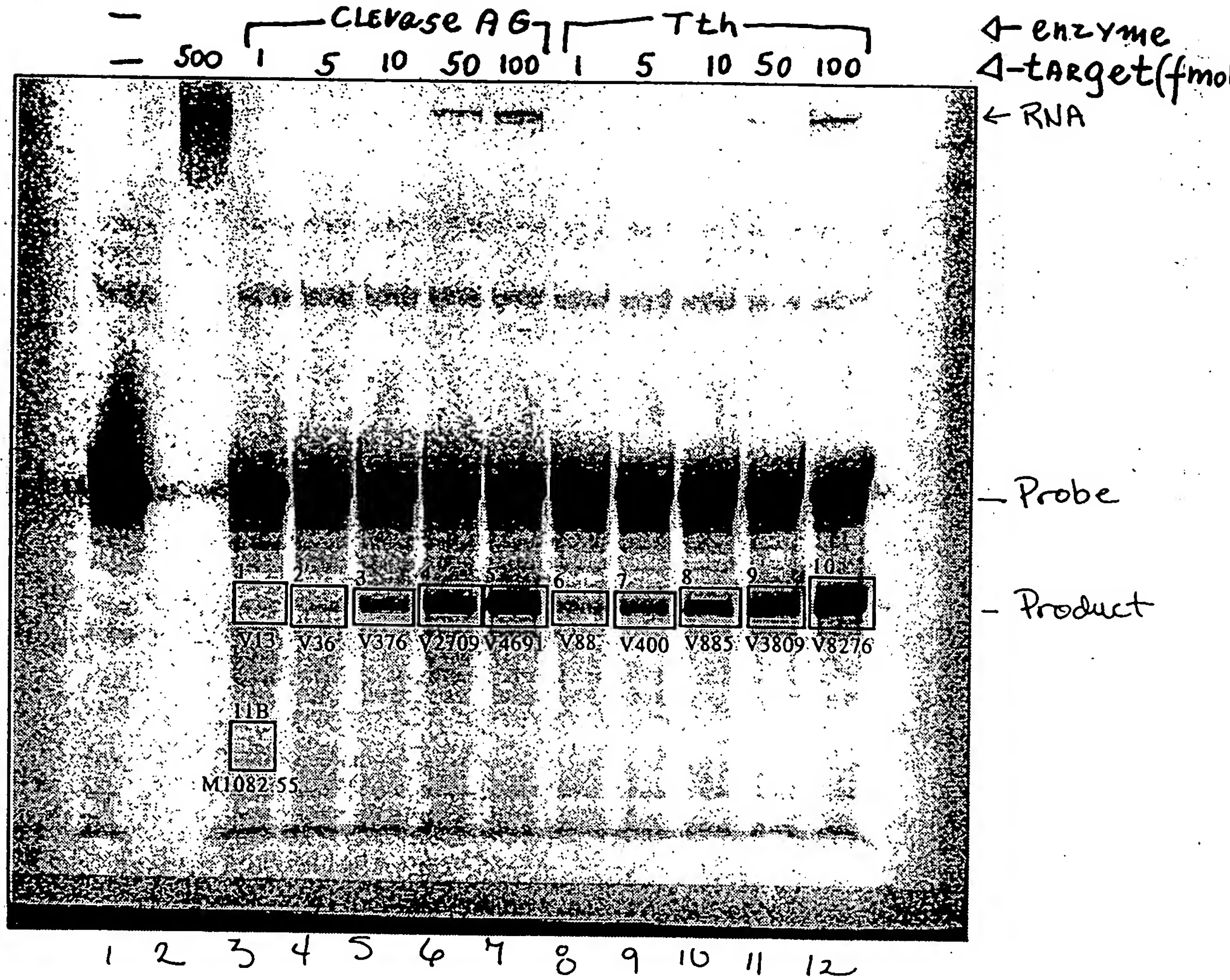
FIGURE 52

FIGURE 53



65

FIGURE 54



Clevase AG
Tth
500
1
5
10
50
100
1
5
10
50
100
enzyme
target(fmol
RNA
Probe
Product
V13
V36
V376
V2709
V4691
V88
V400
V885
V3809
V8276
11B
M1082.55
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 55

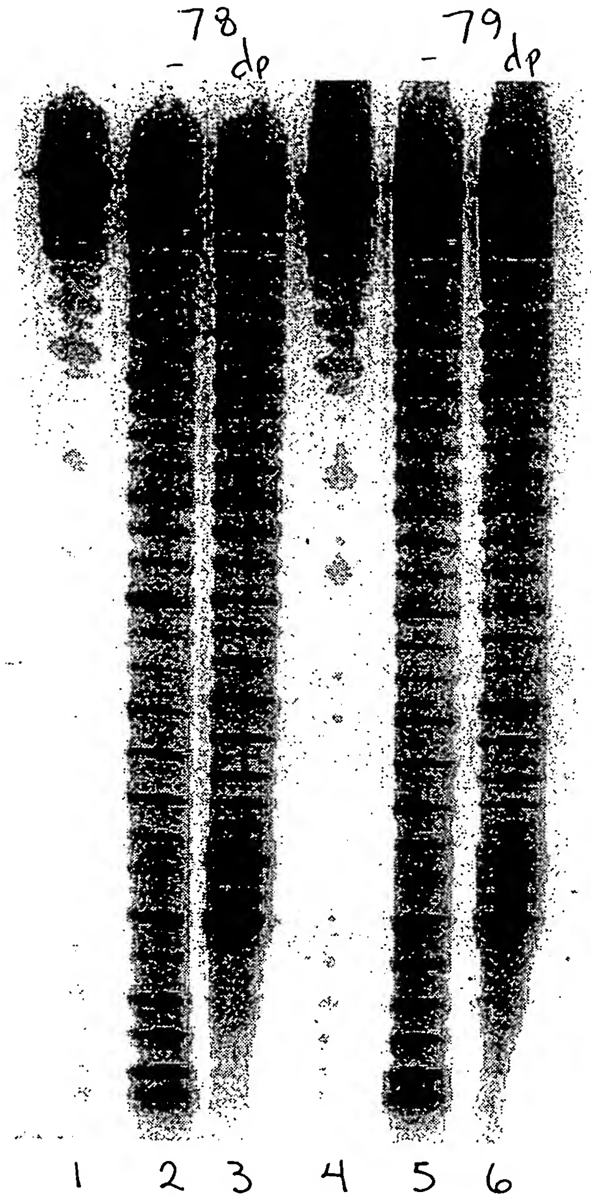


FIGURE 56

70 (C10 aminoT's)
74 (C6 amino T's)

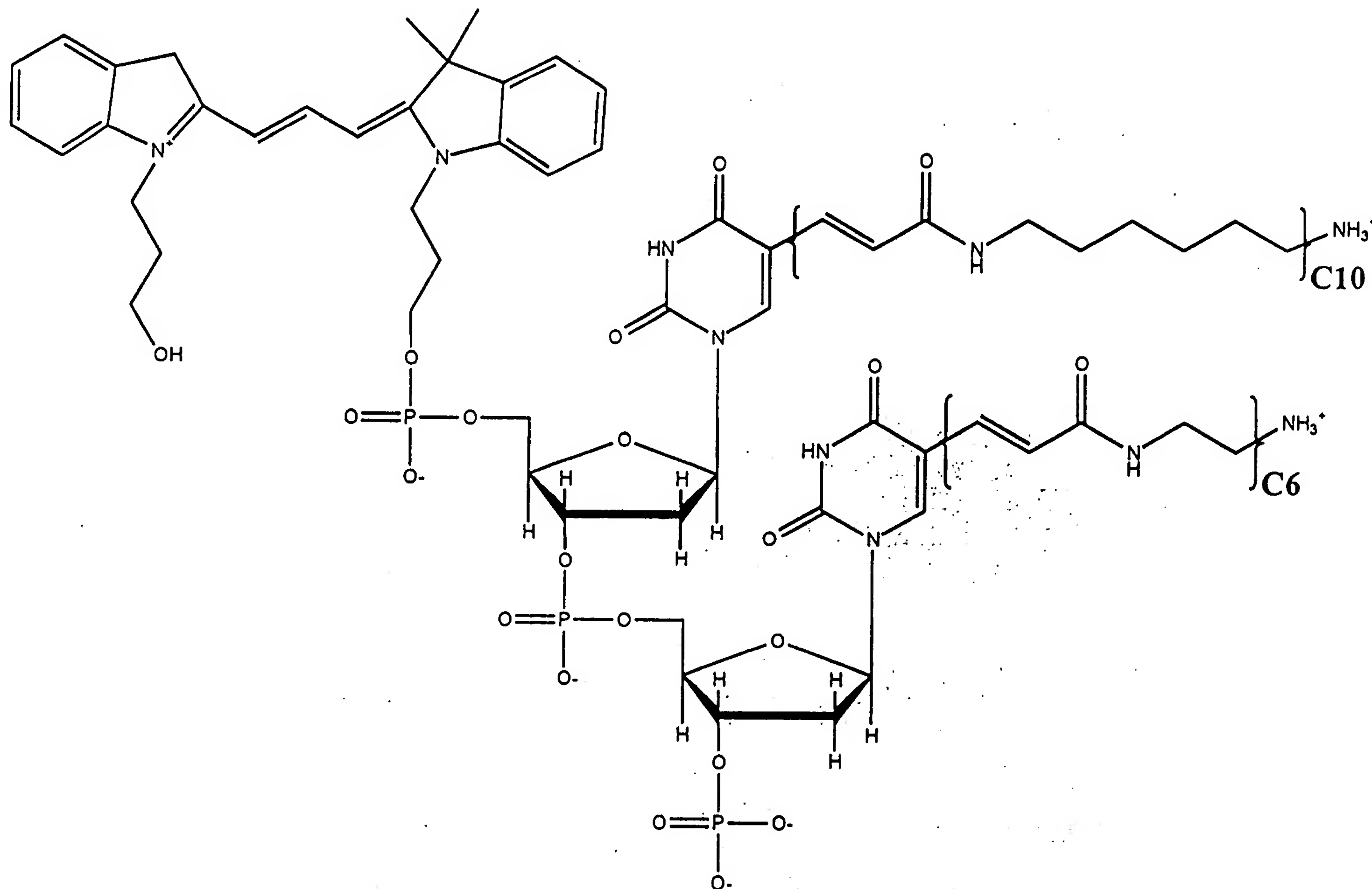


FIGURE 57

75

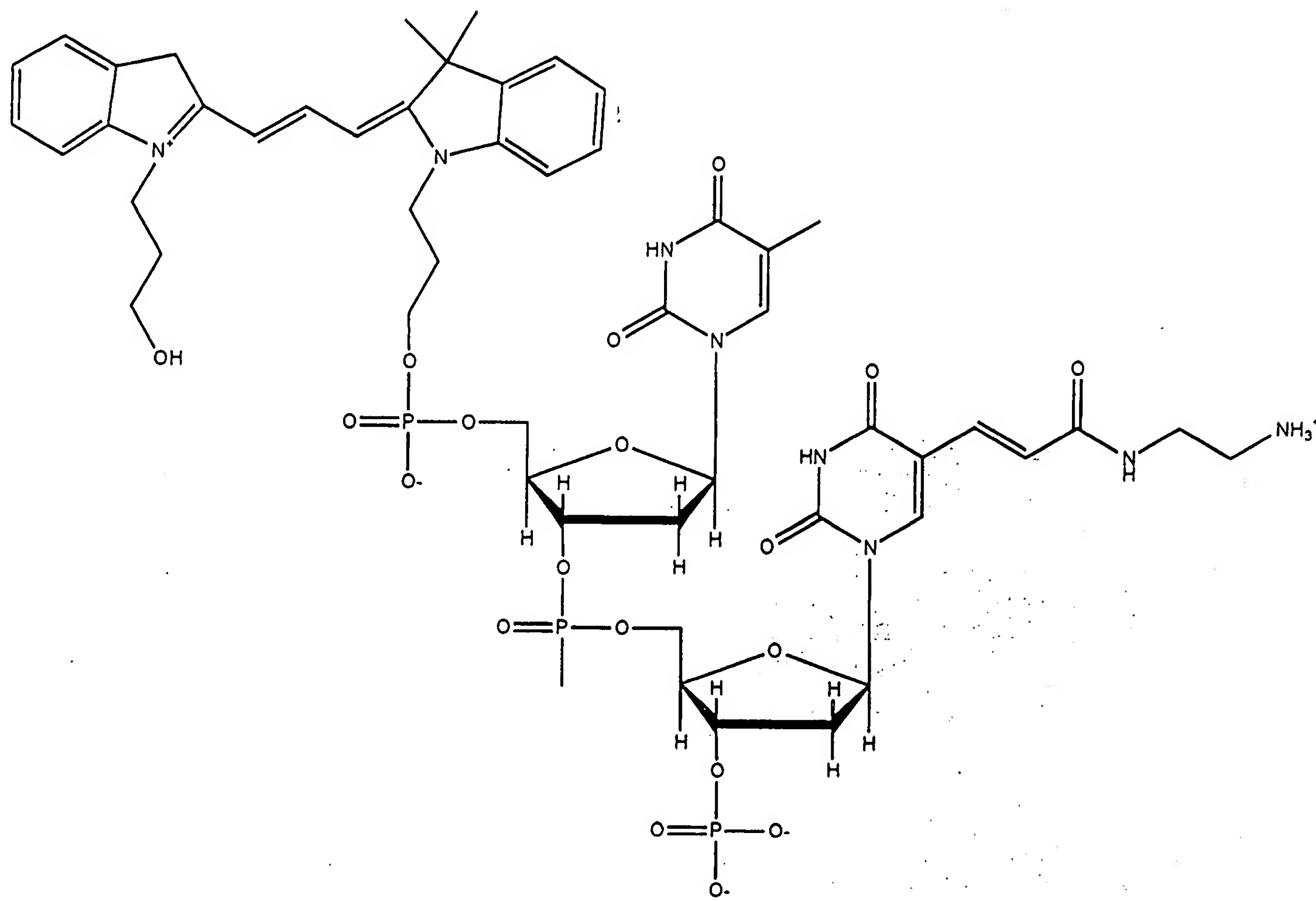


FIGURE 58

76

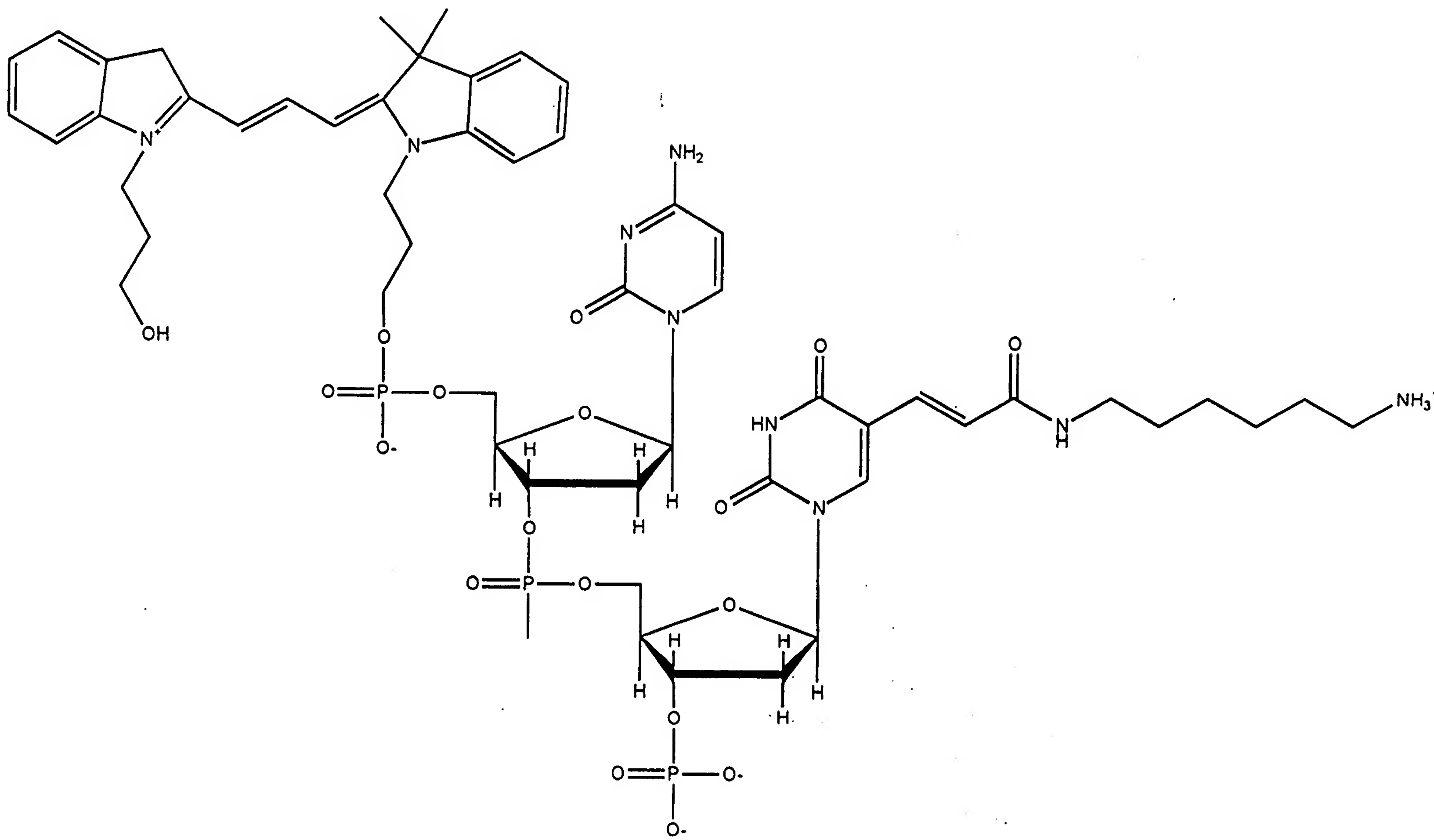


FIGURE 59

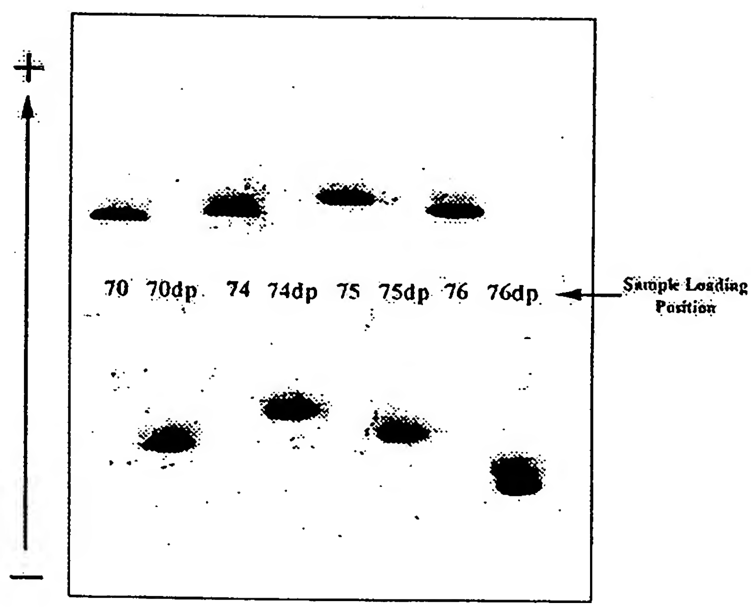


FIGURE 60

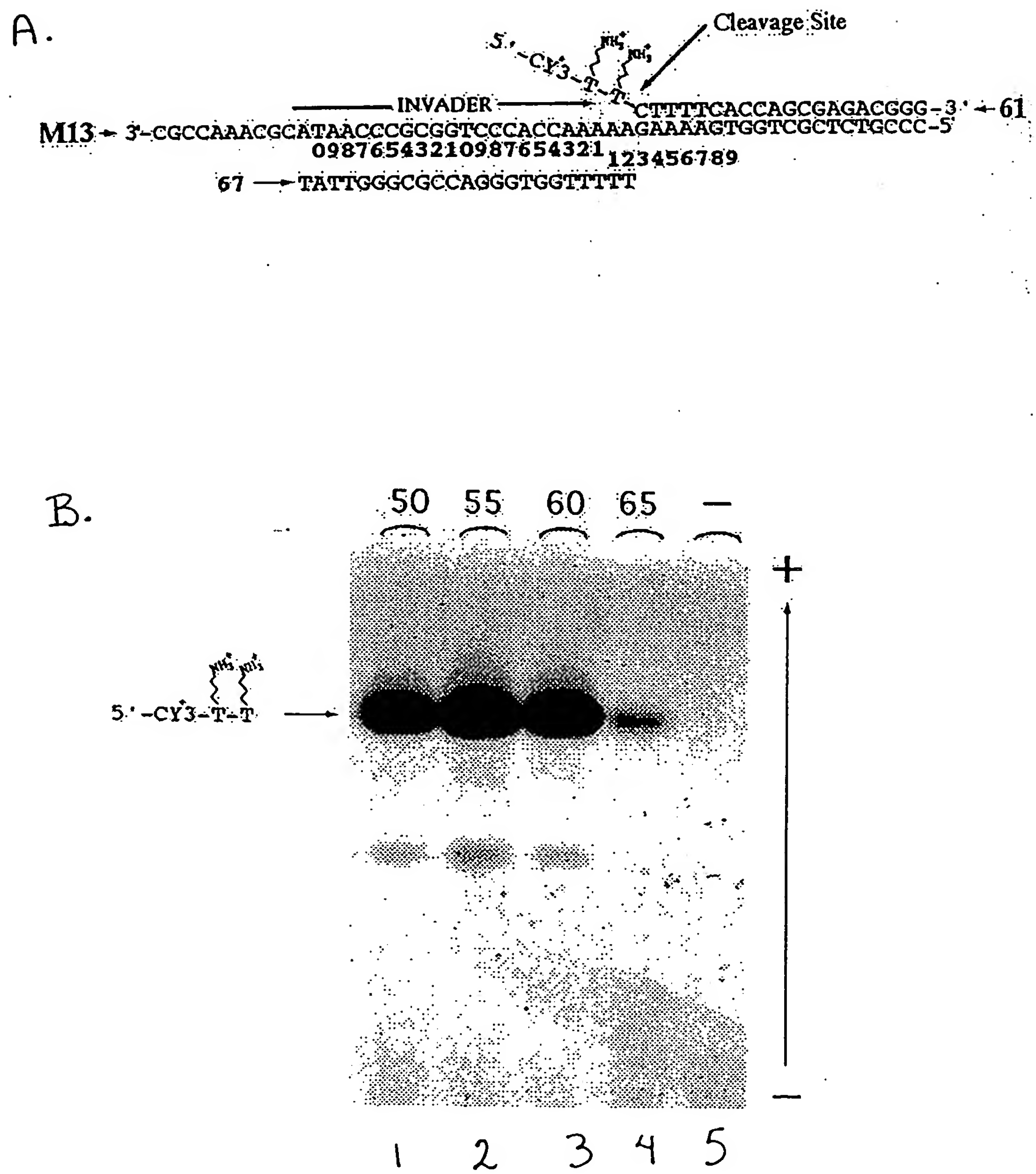

A.
Cleavage Site
5'-CY3-T-T
INVADER
CTTTTCACCAGCGAGACGGG-3' ← 61
M13 → 3'-CGCCAAACGCATAACCCGCGGTCCCACCAAAAAGAAAAGTGGTCGCTCTGCCC-5'
09876543210987654321 123456789
67 → TATTGGGCGCCAGGGTGGTTTTT
B.
50 55 60 65 —
+
5'-CY3-T-T
—
1 2 3 4 5

FIGURE 61

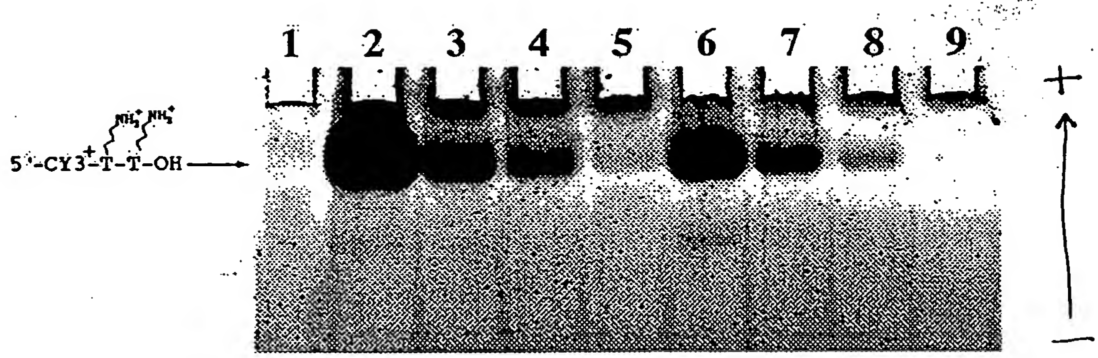


FIGURE 62

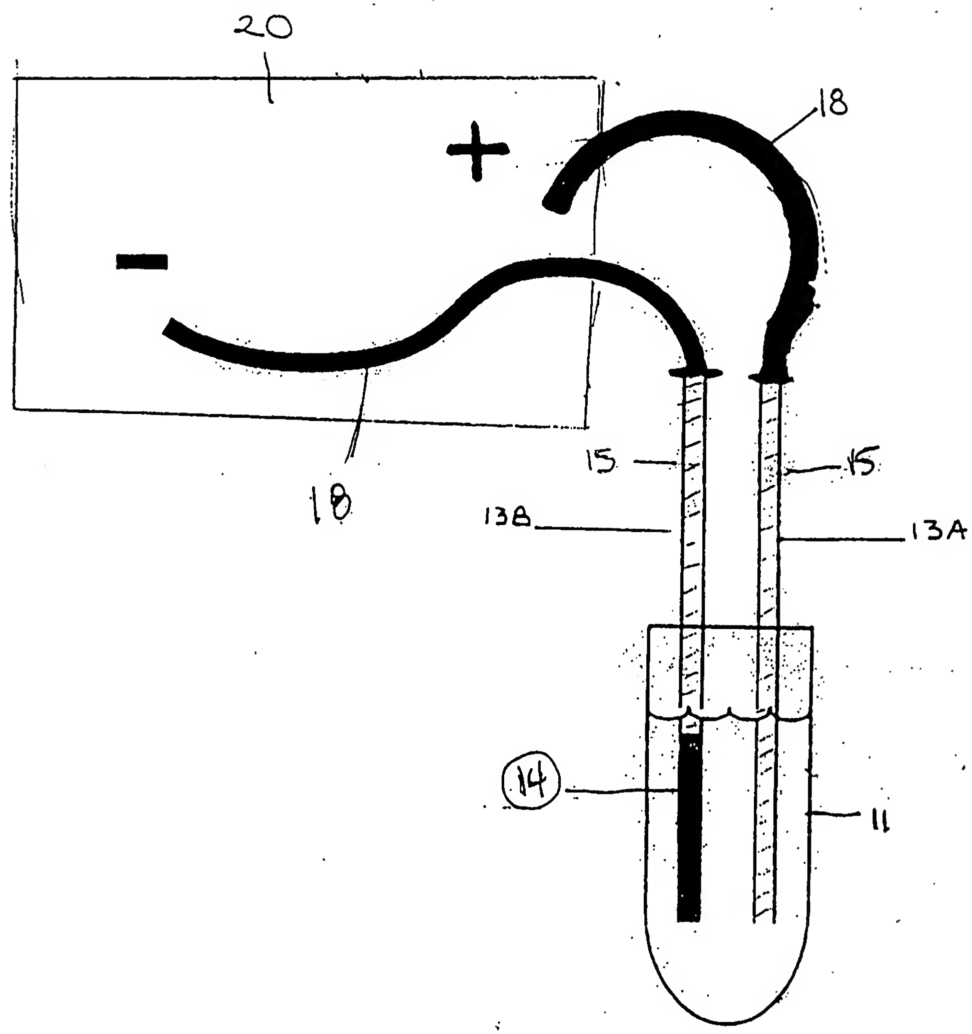


FIGURE 63

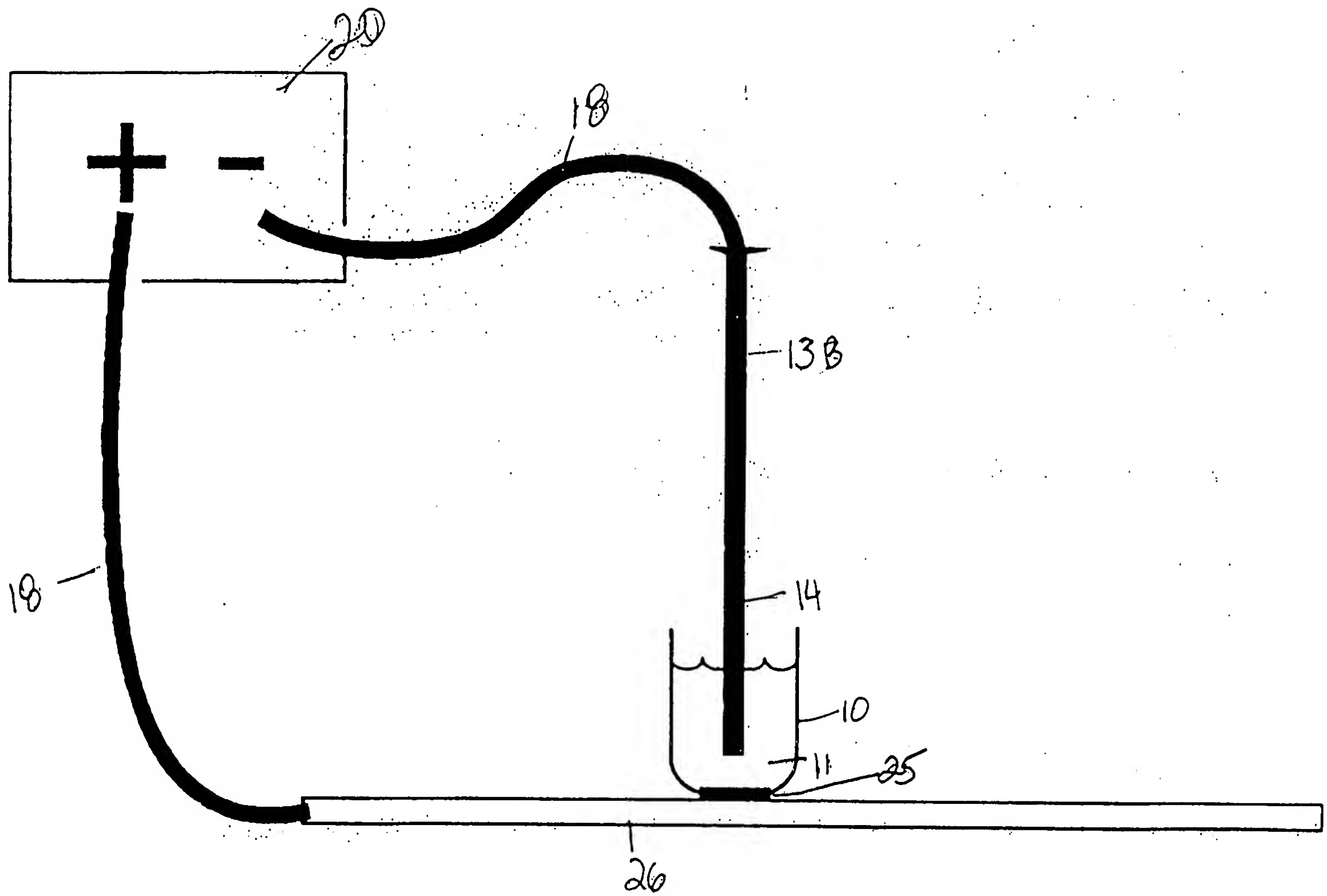


FIGURE 64

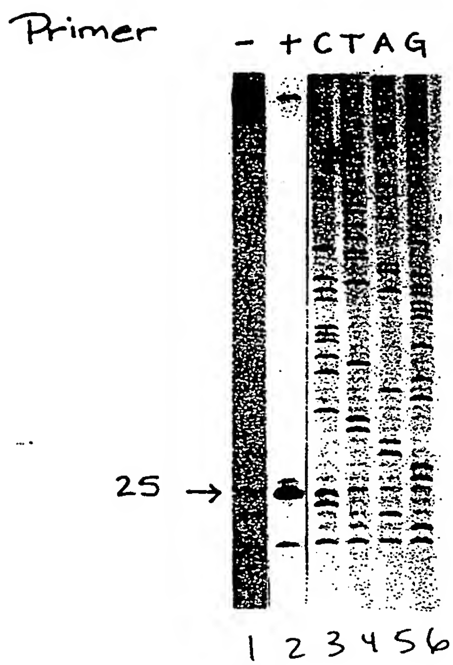
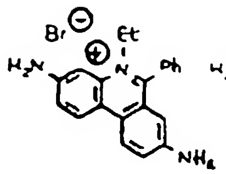


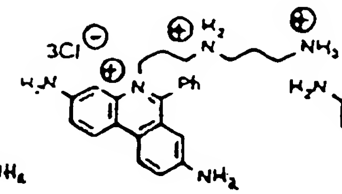
FIGURE 65

```
a
                    AGAAAGGA
5'                  AGAAAGGAAGGGAAGAAAGCGAAAGG 3'
3' CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'

                    AGAAAGGA
5' GCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGG 3'
3' CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'

b
5'                  CAG AAGGAAGGGAAGAAAGCGAAAGG 3'
3' CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'

                    CAGAAAGGA
5' GCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGG 3'
3' CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'

c
5'                  CAGGG GGAAGGGAAGAAAGCGAAAGG 3'
3' CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'

                    CAGGGGGA
5' GCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGG 3'
3' CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'

d
5'                  CAGGGTACAGGGAAGAAAGCGAAAGG 3'
3' CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'

                    CAGGGTAC
5' GCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGG 3'
3' CGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCC 5'
```

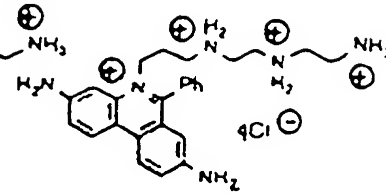
FIGURE 66



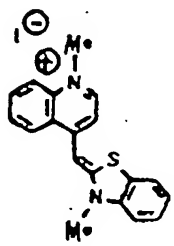
Ethidium Bromide



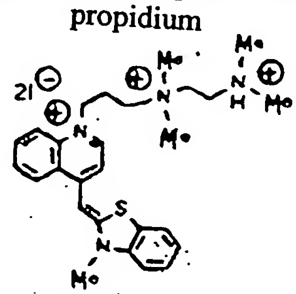
(1,3-propanediamino)-propidium



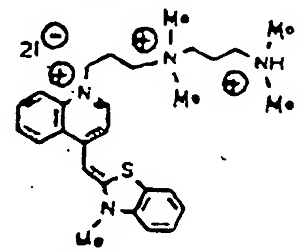
(diethylenetriamino)-propidium



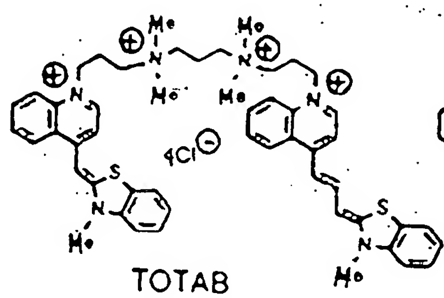
Thiazole Orange



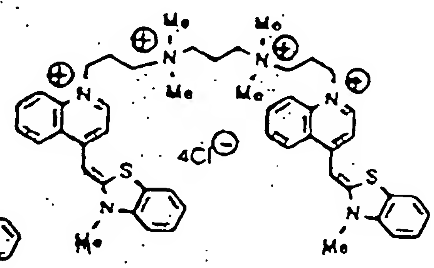
(N-N'-tetramethyl-1,2-ethanediamino)-propyl thiazole orange



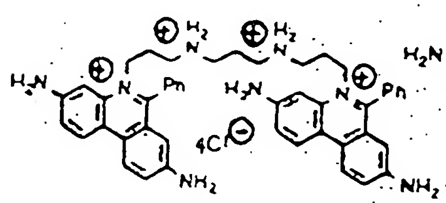
(N-N'-tetramethyl-1,3-propanediamino)-propyl thiazole orange



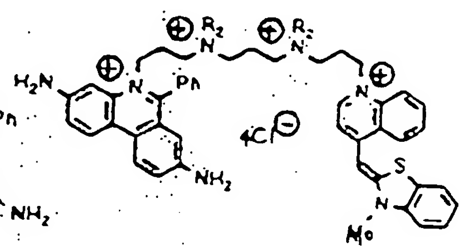
TOTAB



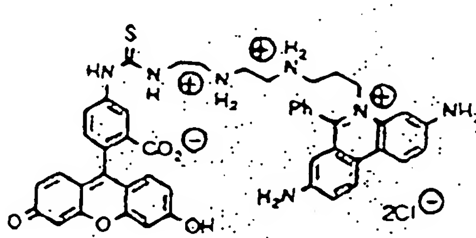
TOTO



EthD



TOED1 (R = H)
TOED2 (R = CH_3)



FED